BMC Microbiology

RESEARCH Open Access

Phenotypic and genotypic determination of resistance to common disinfectants among strains of *Acinetobacter baumannii* producing and non-producing biofilm isolated from Iran

Mohammad Rostamani[1,2], Mehdi Bakht[1,2], Sara Rahimi[1,2], Safar Ali Alizadeh[1], Raana Kazemzadeh Anari[1,2], Mohadeseh Khakpour[1,2], Amir Javadi[1,3], Fatemeh Fardsanei[1] and Farhad Nikkhahi[1*]

Abstract

Background Nosocomial infections are a global problem in hospitals all around the world. It is considered a major health problem, especially in developing countries. The increase in the patient's stay in hospitals has increased the mortality rate, and consequently, the costs drastically increase. The main purpose of using disinfectants in the hospital environment is to reduce the risk of nosocomial infections. Ethylene diamine tetra acetic acid (EDTA) causes lysis and increases susceptibility to antimicrobial agents in the planktonic form of bacteria. This substance affects the permeability of the outer membrane of bacteria. It also prevents the formation of biofilms by bacteria.

Materials and methods In the current study, 120 isolates of *Acinetobacter baumannii* (*A. baumannii*) were confirmed by phenotypic and genotypic methods. Antibiogram was performed and then the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of isolates against 5% sodium hypochlorite, ethanol %70, sayasept-HP 2%, chlorhexidine 2%, dettol 4/8% were evaluated. In addition, the disinfectant effect was re-evaluated with the mixture of EDTA solution. All isolates were examined for biofilm presence by crystal violet staining method in triplicates and repeated three times for each strain. Also for all isolates detection of efflux pump genes (*Qac-E*, *qacE-Δ1*, *SUG-E*) by PCR technique was done.

Results Antibiogram results of *A. baumannii* showed that 6.7% were Multi-drug-resistant (MDR), and 89.2% were Extensively drug-resistant (XDR) isolates. The highest effect of disinfectants was related to 5% sodium hypochlorite, and the least effect was 70% ethanol. EDTA increases the efficacy of selected disinfectants significantly. The highest prevalence of the efflux pump genes was related to *SUG-E* (95%) and *Qac-E* (91.7%), and, the *qacE-Δ1* gene with 12.5%. The biofilm production rate was 91.3% among all isolates.

Conclusion The best and safest way to disinfect hospital floors and surfaces is to choose the right disinfectants, and learn how to use them properly. In this study, a mixture of disinfectants and EDTA had a significant effect on

*Correspondence:
Farhad Nikkhahi
Farhadnikkhahi@gmail.com

Full list of author information is available at the end of the article

bactericidal activity. it was found that improper use of disinfectants, especially the use of sub-inhibitory dilutions, increases the resistance of bacteria to disinfectants.

Keywords Hospital disinfectants, *Acinetobacter baumannii*, Clinical isolates, Antibiogram, Detergent

Introduction

One of the most important pathogens that are increasingly spreading all around the world is *Acinetobacter* [1, 2]. *Acinetobacter* strains can cause infections, especially in immunocompromised patients. This pathogen is a major cause of skin and soft tissue infections in hospital bacteremia, secondary meningitis, urinary tract infections, and pneumonia, especially ventilator-associated pneumonia. It is also a common cause of wound infections at the surgical site, periodontitis, endocarditis, and intra-abdominal abscess [3]. *A. baumannii* can survive in the intensive care unit (ICU) for up to four weeks. This could be due to the production of biofilm, which in turn contaminates hospitalized patients. Lipopolysaccharides (LPS), vesicles and proteins, polysaccharide capsules, phospholipases, proteases, outer membrane purines, biofilm, efflux pump, and iron absorption systems are the most important factors of resistance of this bacterium, which protect bacteria against biocides or drying on hospital surfaces [4]. Today, the dramatic increase in the prevalence of multidrug-resistant *A. baumannii* (MDR, XDR, and PDR) has attracted special attention to this opportunistic pathogen [5]. As a result, it highlights the importance of controlling and preventing infection [6]. The World Health Organization (WHO) states that more than 80% of human infections are caused by biofilms. Biofilm formation can cause several medical problems, including infection of external instruments such as cutters, pharyngeal tubes, and contact lenses, as well as infection of living tissues such as endocarditis, wound infection, vaginitis, colitis, gingivitis, and lung epithelium infection, especially patients with cystic fibrosis [7, 8]. The efflux pumps play an important role in biofilm formation [9]. Inactivation of efflux pumps with reduced biofilm formation has been observed in many bacteria such as *E. coli*, *Salmonella enterica*, and *typhimurium* [10].

Antiseptics and disinfectants have been used in medical centers to disinfect various medical surfaces and devices [11]. The potential emergence of biocidal-resistant bacteria and the possible link between biocidal-resistance and antibiotics are currently major topics of global concern [11].

One of the mechanisms of resistance to antiseptics and disinfectants is the expression of efflux pump systems including *qac* genes. *A. baumannii*, like other gram-negative bacteria, has multi-drug efflux pump systems. *qac* genes are widely amplified in gram-negative bacteria [12].

EDTA increases sensitivity to antimicrobial agents in the planktonic form of bacteria [13]. It is a metal chelator that affects the permeability of the outer membrane of bacteria. This compound cleaves LPS from divalent cations by chelating divalent cations from the outer membrane. It becomes a cell surface and increases the permeability of the outer membrane [5]. It also prevents the formation of biofilms by bacteria [14]. The aim of this study was to evaluate the disinfecting power of syasept HP, sodium hypochlorite, dettol, ethanol alcohol, chlorhexidine separately and mixed with EDTA between strains of *A. baumannii* producing and non-producing biofilm isolated, and determine the prevalence of resistance genes in this bacterium.

Materials and methods

Bacterial isolates

In a cross-sectional study from April 2021 to July 2022, a total of 120 *A. baumannii* isolates were collected. These clinical specimens were isolated from pus/wound, throat swab, nasal swab, sputum, urine, blood culture, and tracheal aspirate. Identification of *A. baumannii* was performed by routine biochemical tests [15]. Isolation tests were confirmed by PCR and *A. baumannii blaOXA-51* gene (Figure S1 (Supplementary Fig. 1).

Antimicrobial susceptibility testing

Antibiogram was performed on Muller-Hinton agar (Merck, Darmstadt, Germany). The disc diffusion method was done in accordance with the Clinical and Laboratory Standards Institute (CLSI 2022) guidelines. Antibiotic discs (MAST Diagnostics, Merseyside, UK) were as follow: ampicillin/sulbactam (10/10 µg), ceftazidime (30 µg), ceftizoxime (30 µg), imipenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), cefoxitin (30 µg), piperacillin/tazobactam (100 /10 µg), ceftriaxone (30 µg), meropenem (10 µg), and cefepime (30 µg) (MAST, Merseyside, UK). *Pseudomonas aeruginosa* ATCC 27,853 and *Escherichia coli* ATCC 25,922 were used as reference strains for quality control.

The MICs for Colistin were determined using the MIC (micro broth dilution) method, then incubated at 37 °C for 18–24 h. Colistin susceptibility was interpreted according to the CLSI 2020 clinical breakpoints [16].

Extraction of total DNA

The bacterial genome was extracted by boiling method. In this method genomic DNA was extracted from a single colony of each isolate, In the next step, a bacterial suspension was prepared in sterile distilled water. And then it was placed in boiling water for ten minutes. Micro tube centrifuged at 10,000 g at 4 ° C for 10 min. The quality and quantity of extracted DNA were evaluated using the Nanodrop instrument and gel electrophoresis (Termo Scientific, Waltham, MA, USA). Finally, the supernatant was transferred to a sterile micro tube to be used as a DNA template for further studies. Prior to PCR amplification, the extracted DNA was preserved at -20 [17]. primers used in this study are shown in Table 1.

Determination of disinfectants MICs and MBCs

In this study, five common disinfectants were tested, including ethanol 70%, sodium hypochlorite 5%, dettol 4.8%, chlorhexidine 2%, and sayasept- HP 2%. MICs and MBCs of all the mentioned disinfectants were evaluated using the broth micro-dilution method. The lowest concentration that inhibits bacterial growth is reported as the MIC. Then, from the three final dilution wells that are transparent 100 µl of each disinfectant was cultured in Muller Hilton agar and if 99.9% of the bacteria had no growth after 48 h at 37° C it is determined as the MBC [18].

Investigating the synergistic effect of selective disinfectants and EDTA

In this method, the selected disinfectants were mixed with EDTA 17% in a one-to-one ratio. Then for all isolated strains MIC and MBC were determined with the new mixture. The obtained results were compared with the previous results and their synergistic effect was investigated.

Assessment of biofilm formation capacity

The biofilm-forming ability was determined using the crystal violet staining method in triplicates and repeated three times for each strain, as previously described [19–21]. LB medium was used as a negative control and *P. aeruginosa* ATCC 27,853 was used as a positive control.

Table 1 Primer sequences used for detection of antiseptic resistance *A. baumanni*

Gene	Amplicon, bp	Sequence
Qac-E	240	Forward: AATTGCGATTGCTTGTGAAG Reverse: CAGGCAGCCAAGTCTAAATG
QacEΔ1	202	Forward: TAGCGAGGGCTTTACTAAGC Reverse: ATTCAGAATGCCGAACACCG
SUG-E	109	Forward: AGCGGCAATCATTCTCATC Reverse: CCTTGGTACTGCTTATACGG
Oxa51	440	Forward-ATCTCTACCTCGCCATTG Reverse-TCGAGCTTCTGCTGGTAG

The bacterial isolates were inoculated with turbidity equal to 0.5 McFarland (1.5×10^8 CFU mL^{-1}). A 200-µL suspension was incubated in each well at 35°± 2 C. After 48 h, the wells were washed three times with phosphate buffer. Following incubation with 1% crystal violet dye (200 µL/well) at 25°C for 20 min, the wells were washed three times with phosphate buffer and dried. Finally, Ethanol 95% (200 µL/well) was added, and optical absorbance was measured at 550 nm (Thermo Scientific GmbH, Driesch, Germany). Biofilm formation was classified into four different groups using the following formulas: If OD < ODc, the biofilm was not formed (negative), If ODc < OD < 2xODc, the biofilm was weak, if 2xODc < OD < 4xODc, the biofilm was moderate. If 4xODc < OD, the biofilm was strong [22].

Statistical analysis

Descriptive statistics were used to measure the characteristics of the study. Pearson chi-square or Fisher's exact test and McNemar's test was used to determine significant differences between proportion. P values of <0.05 were considered significant. Statistical analysis was performed by using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Bacterial isolates

A total of 120 *A. baumannii* isolates were collected from clinical specimens 28 were recovered from blood)23.3%, (22 from wound swabs (18.3%), 36 from urine (30%), and 34 were sputum and secretions collected from chest tubes (chest catheters) (28.4%).

Antimicrobial susceptibility testing

The study showed that the lowest resistances were related to colistin and doxycycline with 27 (22.5%) isolates and the highest resistance was related to cefoxitin with 120 (100%) isolates.

The rate of resistance to other antibiotics is as follows: From the carbapenem family imipenem 113 (94.2%) and meropenem 115 (95.8%) isolates. From the cephalosporin family cefepime 116 (96.7%), ceftriaxone 117 (97.5%), ceftazidime 116 (96.7%), ceftizoxime 118 (98.3%) isolates. From aminoglycoside family tobramycin 104 (86.7%) and gentamicin 106 (88.3%) isolates. From sulfonamides trimethoprim-sulfamethoxazole 110 (91.7%) isolates. From quinolones ciprofloxacin 114 (95%) and levofloxacin 115 (95.8%) isolates. From penicillin family ampicillin-sulbactam 116 (96.7%) and piperacillin/tazobactam 117 (97.5%) isolates were resistant (Table 2(. In this study, 117 MDR strains and 109 XDR strains were among the isolates.

Table 2 Antibiotic susceptibility of clinical isolates of *A. baumanii*

Antibiotic	Susceptible *N* (%)	Intermediate *N* (%)	Resistant *N* (%)
Ceftazidime(30 µg)	3 (2.5)	1 (0.8)	116 (96.7)
Ampicillin/sulbactame (10/10 µg)	4 (3.3)	0	116 (96.7)
Meropenem(10 µg)	5 (4.2)	0	115 (95.8)
Ciprofloxacin(5 µg)	6(5)	0	114(95)
Imipenem(10 µg)	7 (5.8)	0	113 (94.2)
Gentamicin(10 µg)	10 (8.3)	4 (3.3)	106 (88.3)
Doxycycline(30 µg)	91(75.8)	2(1.7)	27(22.5)
Tobramycin(10 µg)	13 (10.8)	3 (2.5)	104 (86.7)
Levofloxacin(5 µg)	5 (4.2)	0	115 (95.8)
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	7 (5.8)	3 (2.5)	110 (91.7)
Cefepime(30 µg)	4 (3.3)	0	116 (96.7)
Ceftriaxone(30 µg)	3(2.5)	0	117(97.5)
Piperacillin/tazobactam (100 µg/10 µg)	3 (2.5)	0	117 (97.5)
Cefoxitin (30 µg)	0	0	120 (100)
Ceftizoxime (30 µg)	2 (1.7)	0	118 (98.3)
Colistin MIC	93(77.5)	0	27(22.5)

Detection of efflux pump genes (*qac-E*, *qacE-Δ1*, *sug-E1*) by PCR technique

All isolates were evaluated for resistance genes. Of 120 *A. baumannii* isolates, 110 (91.7%) harbored the *qac-E* gene (Figure S2 (Supplementary Fig. 2). The prevalence of the *sug-E1* gene was 114 (95%) (Figure S3) The frequency of the *qacE-Δ1* gene was 15 (12.5) (Figure S4).

Determination of MIC and MBC of disinfectant

In this study, the most effective antiseptics respectively was sodium hypochlorite 5%, chloroxylenol (Dettol) 4.8%, sayasept-HP 2%, chlorhexidine 2%, and ethanol 70%. Ethanol had the least antiseptic effect. According to Table 3, the MBC of sodium hypochlorite was 1/16 dilutions and ethanol alcohol was 1/4 dilutions, which prevented the growth of all isolates.

Synergistic effect of disinfectants with EDTA

During this study, a synergistic effect of biocides with EDTA was significantly observed against *A. baumannii*. The most synergistic effect after adding equal proportions of biocides and EDTA 17%, was related to ethanol, sodium hypochlorite, and HP syasept, chlorhexidine respectively. Dettol shows the least synergistic effect (Figs. 1, 2 and 3).

According to Figs. 1 and 2, all biocides inhibited most isolates at dilutions of 1/32, 1/64, and 1/128. After mixing with EDTA the growth of most isolates was inhibited with dilutions of 1/128, 1/256, and 1/512, indicating an increase in MIC with the mixture EDTA ($PV \leq 0.05$).

Table 3 Distributions of MIC and MBC of various biocides by micro broth dilution technique

	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Biocides MIC							
Ethanol 70%	19(15.8%)	21(17.5%)	52(43.3%)	28(23.3%)	-	-	-
Chlorhexidine 2%	-	24 (20%)	24 (20%)	55 (45.8%)	17 (14.2%)	-	-
Sayasept-HP 2%	-	18 (15%)	31 (25.8%)	49 (40.8%)	22 (18.3%)	-	-
Dettol 4.8%	-	-	40 (33.3%)	40 (33.3%)	35 (29.2%)	5 (4.2%)	
Sodium hypochlorite 5%	-	-	11 (9.2%)	43 (35.8%)	42 (35%)	24 (20%)	-
Biocides + EDTA MIC							
Ethanol 70%+EDTA	-	-	23(19.2%)	44 (36.7%)	51 (42.5%)	2 (1.7%)	-
Chlorhexidine 2%+EDTA	-	-	14 (11.7%)	39 (32.5%)	48 (40%)	19 (15.8%)	-
Sayasept-HP 2%+EDTA	-	-	10 (8.3%)	37 (30.8%)	50 (41.7%)	23 (19.2%)	-
Dettol 4.8%+EDTA	-	-	11 (9.2%)	29 (24.2%)	36 (30%)	20 (16.7%)	24 (20%)
Sodium hypochlorite 5%+EDTA	-	-	-	11 (9.2%)	28 (23.3%)	46 (38.3%)	35(29.2%)
Biocides MBC							
Ethanol 70%	24 (20%)	35 (29.2)	48 (40%)	13 (10.8%)	-	-	-
Chlorhexidine 2%	-	24(20%)	37(30.8%)	44(36.7%)	15(12.5%)	-	-
Sayasept- HP 2%	-	19 (15.8%)	41 (34.2%)	44 (36.7%)	16 (13.3%)	-	-
Dettol 4.8%	-	8 (6.7%)	34 (28.3%)	42 (35%)	36 (30%)	-	-
Sodium hypochlorite 5%	-	-	14 (11.7%)	55 (45.8%)	37 (30.8%)	14 (11.7%)	-
Biocides + EDTA MBC							
Ethanol 70%+EDTA	-	18 (15%)	35 (29.2%)	60 (50%)	7 (5.8%)	-	-
Chlorhexidine 2%+EDTA	-	-	25(20.8%)	48(40%)	39(32.5%)	8(6.7%)	-
Sayasept- HP 2%+EDTA	-	-	19 (15.8%)	49 (40.8%)	33 (27.5%)	19 (15.8%)	-
Dettol 4.8%+EDTA	-	-	19 (15.8%)	48 (40%)	28 (23.3%)	25 (20.8%)	-
Sodium hypochlorite 5%+EDTA	-	-	-	17 (14.2%)	43 (35.8%)	42 (35%)	18 (15%)

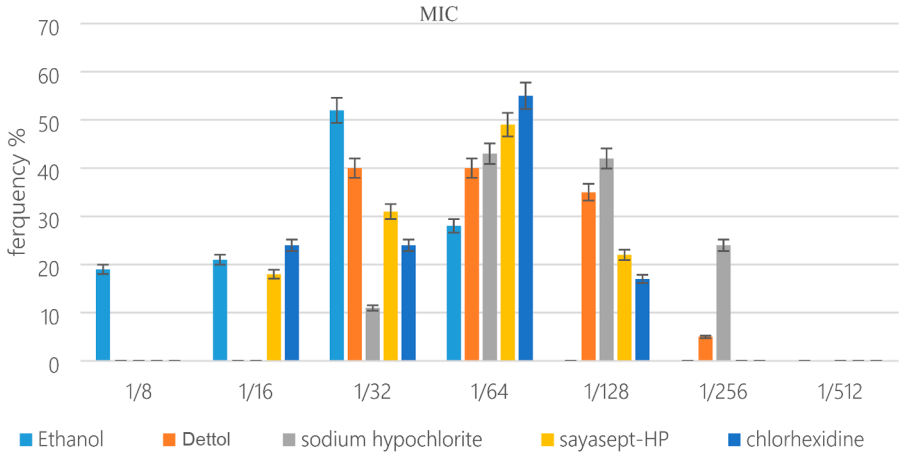

Fig. 1 Diagram of MIC disinfectants at different concentrations

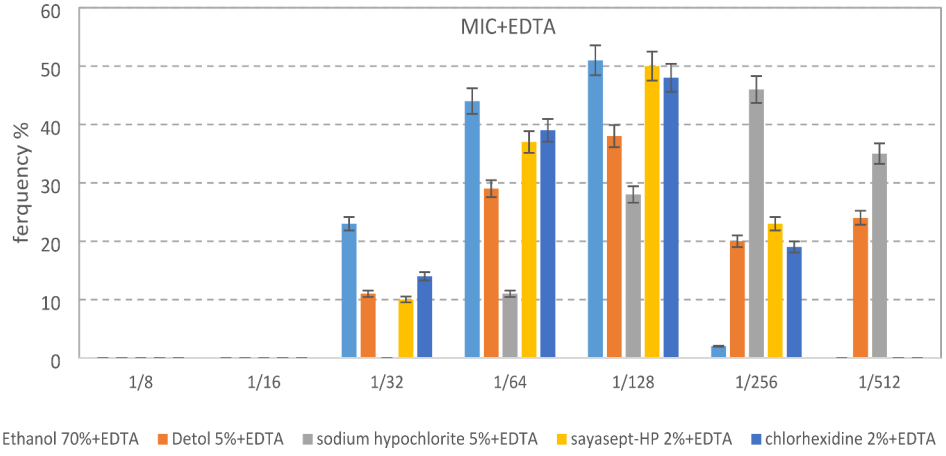

Fig. 2 Diagram of MIC disinfectant + EDTA at different concentrations

Assessment of biofilm formation capacity

In this study, clinical isolates of *A. baumannii* have high potency in biofilm formation. From 120 isolates that phenotypically were tested, 60 isolates (50%) produced strong biofilm, 34 (28.3%) moderate, 16 (13.3%) weak, and 10 (8.3%) had no biofilm production. Biofilm results according to the type of clinical sample are shown in Table 4. The results indicated that strong and moderate biofilm formation isolates need higher concentrations (MIC and MBC) of disinfectant for killing.

Discussion

One of the most important issues in bacteriological sciences is the resistance of bacteria to antibiotics and disinfectants. This phenomenon may explain the reason for not responding to treatment and prevention in controlling the causes of various diseases. In recent years efforts have been done to improve infection control in hospitals, which has led to the overuse of disinfectants, which has had detrimental effects on patients [23]. Nosocomial infection is one of the most serious problems in the world, which has increased the burden of medical expenses and the death of patients every year [24, 25].

In a study conducted in Iran, the results of antibiotic resistance overlapped with our study. Except for gentamicin, which had lower antibiotic resistance than our study [5]. Antibiotic resistance in the present study shows an increase compared to tobramycin and gentamicin with the Zeighami study in 2018, while doxycycline decreased resistance, which may be due to differences in the pattern of antibiotic use and different geographical areas [26].

Biofilm production is a feature of *A. baumannii* that sticks to inanimate surfaces and causes infection to persist and spread among patients. Biofilm production can increase the resistance of this bacterium to antibiotics and disinfectants [27]. The use of biocides against biofilm-producing bacteria causes the low diffusion of biocides into the biofilm, which causes the bacterium to be exposed only to small amounts of biocides and not to penetrate deeper into the biofilm. EDTA prevents the formation of biofilm by bacteria [14]. Based on the results of this study it was found that most isolates produced

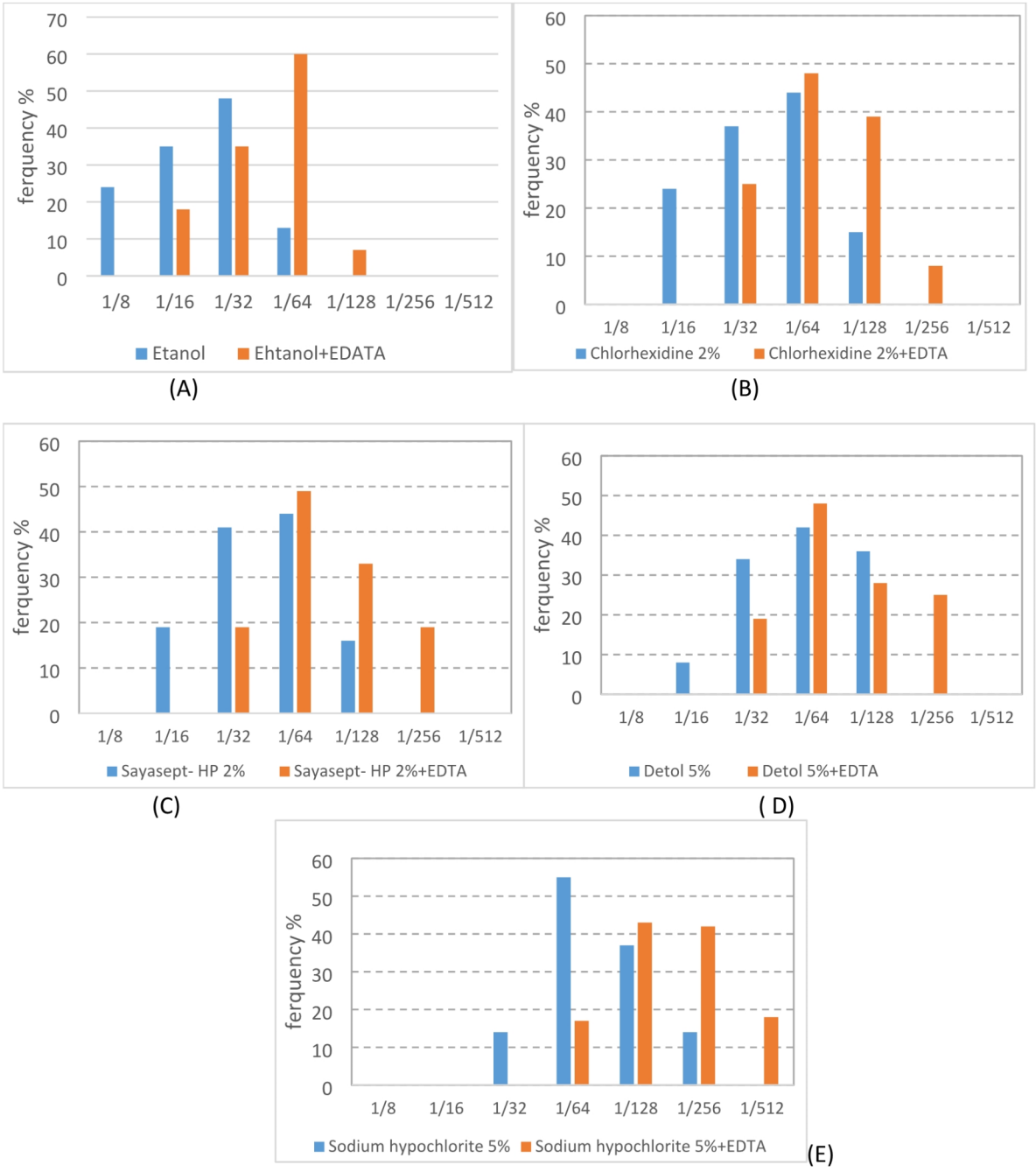

Fig. 3 Evaluation of MBC of disinfectant alone and in combination with EDTA

biofilm (91.7%) and the highest antibiotic resistance was related to isolates that produced strong and intermediate biofilm. In another study, it was found that 92% of the isolates produced biofilm and the highest antibiotic resistance was related to these isolates, which overlaps with our study [28]. Mahdavi et al. performed a study on disinfectants including sodium hypochlorite, propanol alcohol, hydrogen peroxide, and glutaraldehyde in bacterial biofilms. The results showed that bacteria which produce biofilm were more resistant to disinfectants. Sodium hypochlorite had the highest effect on biofilm producer bacteria, and glutaraldehyde had the highest lethal effect on the planktonic form [29]. In another survey performed from 2010 to 2013 on 272 *A. baumannii*

Table 4 Biofilm frequency table among *A. baumannii* isolates in different clinical specimens

Sample type	Strong biofilm	Moderate biofilm	Weak biofilm	Without biofilm
Urine	18	10	6	2
Blood	12	8	5	3
Wound	16	11	4	3
Sputum, chest Catheters	14	5	1	2
Total	60	34	16	10

isolates, 91% of isolates produced biofilm which overlaps with our study [30]. A study showed that 85.9% of *A. baumannii* isolates produce biofilm which was highly resistant to antibiotics [31]. Reduce sensitivity to disinfectants are on the rise among bacteria due to overuse and misuse of detergents [20, 21].

SMR proteins from the family of carriers of multidrug resistance in bacteria are a family of proton-dependent efflux pumps and are divided into three groups: SUG, SMP, and PSMR. *qacE-Δ1*, *qacE* genes belong to the SMP subgroup and have been identified on the plasmids and integrons of many drug-resistant gram-negative and gram-positive bacteria [32]. SUG that is one of the main classes of the SMR family, in bacteria resulted in resistant to the quaternary cation compound [33]. Our study was in line with another study conducted in 2017, and the result showed that 10 isolates (45.5%) had *qacE* gene and 15 isolates (68.1%) had *qacE-Δ1* [34]. Another survey performed in 2018, reported the prevalence of *qacE-Δ1* (37.6%) and *sugE* (71.5%) genes that compared to our results, *qacE-Δ1* gene was lower and *sugE* was higher [35].

A comparison of studies showed that the prevalence of *qacE* and *SugE* genes have increased compared to previous studies, while the frequency of *qacE-Δ1* gene has been lower, which can be inferred that this may be due to differences in disinfection resistance patterns and geographical area differences. In our study after alcohol ethanol 70% and chlorhexidine 2%, which had the least bactericidal effect on *A. baumannii*, sayasept-HP 2%, had less effect on this bacterium than dettol 4.8% and sodium hypochlorite 5%.

Our results in this study showed that the highest inhibitory rate for disinfectants are as follows: alcohol ethanol at a dilution rate of 1/32 (2.187), chlorhexidine with sayasept-HP 2% at a dilution of 1/64 (0.03123 mg/ml), dettol dilution rate of 1/32 and 1/64 (0.15, 0.075 mg/ml), and sodium hypochlorite at dilution of 1/64 (0.078 mg/ml). Since the lack of criteria, there is no clinical interpretation for sensitivity or resistance to biocides. isolates cannot be identified as sensitive or resistant to biocides. Lin fei et al. in 2017 reported the disinfectant performance against *A. baumannii* isolates, sodium hypochlorite, and chlorhexidine had the highest inhibitory dilution of 1/64 (0.078 mg/ml), which is in line with our result [36]. In another study, the highest bactericidal rate (MBC) of chlorhexidine was 0.01 dilution, which in the present study was 0.075 which is in line with our study [37].

According to the results obtained in this study, disinfectant after combination with EDTA had synergism effect on the results of both MIC and MBC. The MIC of alcohol changed from dilution 4.375 to dilution 0.5. The MIC of sayasept, and chlorhexidine changed from dilution 0.25 to 0.125. The MIC of sodium hypochlorite changed from dilution 0.321 to dilution 0.156 with the mixture of EDTA. Also, dettol in dilution of 0.15 inhibited the growth of 80 isolates, and at the same dilution in combination with EDTA inhibited the growth of 109 isolates ($P<0.05$). In a study conducted in France on the synergistic effect of EDTA with antibiotics, the synergistic effect of antibiotics combined with EDTA against bacterial biofilm producers was observed [38].

Conclusion

This study showed that *A. baumannii* has a high ability to produce biofilms on medical surfaces and instruments. Therefore, it is necessary to prevent the formation of biofilm and remove it from medical devices. Accordingly, the use of EDTA with the mixture of disinfectants has a significant impact on reducing biofilm production and as a result, nosocomial infections. Based on this study appropriate use of disinfectants at proper concentrations for different species of bacteria should be addressed to avoid inducing resistance mechanisms in bacteria. Also, the field of study using EDTA in combination with disinfectants should be addressed.

Abbreviations

MDR Multidrug-resistant
XDR Extensively drug-resistant
MIC Minimum inhibitory concentration
MBC Minimum bactericidal concentration
WHO World Health Organization
EDTA Ethylene-diamine-tetra acetic acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03484-1.

Supplementary Material 1

Acknowledgements

We gratefully thank the four educational hospitals in Qazvin, Iran (boali, Ghods, Kosar, and velayat). This work would not have been possible without their support of them.

Author contributions

Farhad Nikkhahi and Safar Ali Alizadeh designed the study, Mohammad Rostamani, Mehdi Bakht, and Raana Kazemzadeh Anari performed the study. Mehdi Bakht, Mohammad Rostamani, Sara Rahimi, and Mohadeseh khakpour wrote the manuscript. Amir Javadi performed statistical work on the

article. Fatemeh Fardsanei revised the manuscript. All authors reviewed the manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability
All data and material are available upon request to correspondence author.

Declarations

Ethics approval and consent to participate
The current study was performed by approval of the Ethics Committee of Qazvin Medical University with approval number IR.QUMS.REC.1398.155. In addition, the committee approved the utilization of human samples within this study. We confirm that written informed consent to participate was obtained from all of the participants in our study. We acquired permissions and/or licenses to access the clinical patient data used in our research from Qazvin University of Medical Sciences. Hospitals provided the clinical samples. Also, it should be noted that biological samples are handled by the authors in the present study. The adopted methods for handling human samples were carried out in accordance with relevant guidelines and regulations provided in the Declaration of Helsinki. The research protocol was approved by the Research Ethics Committee at the Qazvin Medical University, Iran.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
[2]Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran
[3]Department of Community Medicine, Qazvin University of Medical Sciences, Qazvin, Iran

Received: 13 May 2023 / Accepted: 29 August 2024
Published online: 06 September 2024

References

1. Kumar A, Saini V, Kumar A, Kaur J, Kaur J. Modulation of trehalose dimycolate and immune system by Rv0774c protein enhanced the intracellular survival of Mycobacterium smegmatis in human macrophages cell line. Front Cell Infect Microbiol. 2017;7:289.
2. Li J, Yu T, Luo Y, Peng J-Y, Li Y-J, Tao X-Y, et al. Characterization of carbapenem-resistant hypervirulent *Acinetobacter baumannii* strains isolated from hospitalized patients in the mid-south region of China. BMC Microbiol. 2020;20:1–8.
3. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538–82.
4. Control, CfD, Prevention. *Acinetobacter baumannii* infections among patients at military medical facilities treating injured US service members, 2002–2004. MMWR Morbidity Mortal Wkly Rep. 2004;53(45):1063–6.
5. Sarhaddi N, Soleimanpour S, Farsiani H, Mosavat A, Dolatabadi S, Salimizand H, et al. Elevated prevalence of multidrug-resistant *Acinetobacter baumannii* with extensive genetic diversity in the largest burn centre of northeast Iran. J Global Antimicrob Resist. 2017;8:60–6.
6. Nasiri A, Balouchi A, Rezaie-Keikhaie K, Bouya S, Sheyback M, Al Rawajfah O. Knowledge, attitude, practice, and clinical recommendation toward infection control and prevention standards among nurses: a systematic review. Am J Infect Control. 2019;47(7):827–33.
7. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95–108.
8. Bjarnsholt T. The role of bacterial biofilms in chronic infections. Apmis. 2013;121:1–58.
9. Li X-Z, Nikaido H. Efflux-mediated drug resistance in bacteria. Drugs. 2009;69(12):1555–623.
10. Smith K, Gemmell CG, Hunter IS. The association between biocide tolerance and the presence or absence of qac genes among hospital-acquired and community-acquired MRSA isolates. J Antimicrob Chemother. 2008;61(1):78–84.
11. Lee H-Y, Hsu S-Y, Hsu J-F, Chen C-L, Wang Y-H, Chiu C-H. Risk factors and molecular epidemiology of *Acinetobacter baumannii* bacteremia in neonates. J Microbiol Immunol Infect. 2018;51(3):367–76.
12. Chang Y-C, Shih DY-C, Wang J-Y, Yang S-S. Molecular characterization of class 1 integrons and antimicrobial resistance in Aeromonas strains from foodborne outbreak-suspect samples and environmental sources in Taiwan. Diagn Microbiol Infect Dis. 2007;59(2):191–7.
13. Zarrilli R, Crispino M, Bagattini M, Barretta E, Di Popolo A, Triassi M, et al. Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance. J Clin Microbiol. 2004;42(3):946–53.
14. Apisarnthanarak A, Khawcharoenporn T, Mundy LM. Practices to prevent multidrug-resistant *Acinetobacter baumannii* and methicillin-resistant Staphylococcus aureus in Thailand: a national survey. Am J Infect Control. 2013;41(5):416–21.
15. Krieg NR, Manual H. Systematic bacteriology. Williams Baltim. 1984;1(161):172.
16. Clinical and Laboratory Standard Institute (CLSI). Performance standards for antimicrobial susceptibility testing. M100-S30th. Clinical and laboratory standards institute. 2020.
17. Qi C, Pilla V, Jessica HY, Reed K. Changing prevalence of Escherichia coli with CTX-M–type extended-spectrum β-lactamases in outpatient urinary E. coli between 2003 and 2008. Diagn Microbiol Infect Dis. 2010;67(1):87–91.
18. Nasr AM, Mostafa MS, Arnaout HH, Elshimy AAA. The effect of exposure to sub-inhibitory concentrations of hypochlorite and quaternary ammonium compounds on antimicrobial susceptibility of Pseudomonas aeruginosa. Am J Infect Control. 2018;46(7):e57–63.
19. King LB, Swiatlo E, Swiatlo A, McDaniel LS. Serum resistance and biofilm formation in clinical isolates of *Acinetobacter baumannii*. FEMS Immunol Med Microbiol. 2009;55(3):414–21.
20. Bakht M, Alizadeh SA, Rahimi S, Kazemzadeh Anari R, Rostamani M, Javadi A, et al. Phenotype and genetic determination of resistance to common disinfectants among biofilm-producing and non-producing Pseudomonas aeruginosa strains from clinical specimens in Iran. BMC Microbiol. 2022;22(1):124.
21. Anari RK, Nikkhahi F, Javadi A, Bakht M, Rostamani M, Kelishomi FZ, et al. Evaluation of antibacterial activity of five biocides and the synergistic effect of biocide/EDTA combinations on biofilm-producing and non-producing Stenotrophomonas maltophilia strains isolated from clinical specimens in Iran. BMC Microbiol. 2022;22(1):1–13.
22. Rahimi S, Farshadzadeh Z, Taheri B, Mohammadi M, Haghighi M-A, Bahador A. The relationship between antibiotic resistance phenotypes and biofilm formation capacity in clinical isolates of *Acinetobacter baumannii*. Jundishapur J Microbiol. 2018;11(8).
23. Braoudaki M, Hilton AC. Low level of cross-resistance between triclosan and antibiotics in Escherichia coli K-12 and E. Coli O55 compared to E. Coli O157. FEMS microbiology letters. 2004;235(2):305–9.
24. Mohammadi Bardbari A, Mohajeri P, Arabestani MR, Karami M, Keramat F, Asadollahi S, et al. Molecular typing of multi-drug resistant *Acinetobacter baumannii* isolates from clinical and environmental specimens in three Iranian hospitals by pulsed field gel electrophoresis. BMC Microbiol. 2020;20(1):1–7.
25. Firesbhat A, Tigabu A, Tegene B, Gelaw B. Bacterial profile of high-touch surfaces, leftover drugs and antiseptics together with their antimicrobial susceptibility patterns at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. BMC Microbiol. 2021;21(1):1–13.
26. Shirmohammadlou N, Zeighami H, Haghi F, Kashefieh M. Resistance pattern and distribution of carbapenemase and antiseptic resistance genes among multidrug-resistant *Acinetobacter baumannii* isolated from intensive care unit patients. J Med Microbiol. 2018;67(10):1467–73.
27. Boone RL, Whitehead B, Avery TM, Lu J, Francis JD, Guevara MA, et al. Analysis of virulence phenotypes and antibiotic resistance in clinical strains of *Acinetobacter baumannii* isolated in Nashville, Tennessee. BMC Microbiol. 2021;21:1–12.
28. Babapour E, Haddadi A, Mirnejad R, Angaji S-A, Amirmozafari N. Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. Asian Pac J Trop Biomed. 2016;6(6):528–33.

29. Mahdavi M, KASRA KR, Jalali M. The assessment of disinfectants on various bacterial biofilms. 2008.
30. Qi L, Li H, Zhang C, Liang B, Li J, Wang L, et al. Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in *Acinetobacter baumannii*. Front Microbiol. 2016;7:483.
31. Amin M, Navidifar T, Shooshtari FS, Rashno M, Savari M, Jahangirmehr F, et al. Association between biofilm formation, structure, and the expression levels of genes related to biofilm formation and biofilm-specific resistance of Acinetobacter baumannii strains isolated from burn infection in Ahvaz, Iran. Infect drug Resist. 2019;12:3867.
32. Harrison JJ, Turner RJ, Joo DA, Stan MA, Chan CS, Allan ND, et al. Copper and quaternary ammonium cations exert synergistic bactericidal and antibiofilm activity against Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2008;52(8):2870–81.
33. Cruz A, Micaelo N, Félix V, Song J-Y, Kitamura S-I, Suzuki S, et al. sugE: a gene involved in tributyltin (TBT) resistance of Aeromonas molluscorum Av27. J Gen Appl Microbiol. 2013;59(1):39–47.
34. Gomaa FAM, Helal ZH, Khan MI. High prevalence of blaNDM-1, blaVIM, qacE, and qacEΔ1 genes and their association with decreased susceptibility to antibiotics and common hospital biocides in clinical isolates of *Acinetobacter baumannii*. Microorganisms. 2017;5(2):18.
35. Sun Y, Hu X, Guo D, Shi C, Zhang C, Peng X, et al. Disinfectant resistance profiles and biofilm formation capacity of Escherichia coli isolated from retail chicken. Microb Drug Resist. 2019;25(5):703–11.
36. Lin F, Xu Y, Chang Y, Liu C, Jia X, Ling B. Molecular characterization of reduced susceptibility to biocides in clinical isolates of *Acinetobacter baumannii*. Front Microbiol. 2017;8:1836.
37. Lanjri S, Uwingabiye J, Frikh M, Abdellatifi L, Kasouati J, Maleb A, et al. In vitro evaluation of the susceptibility of *Acinetobacter baumannii* isolates to antiseptics and disinfectants: comparison between clinical and environmental isolates. Antimicrob Resist Infect Control. 2017;6(1):1–7.
38. Lebeaux D, Leflon-Guibout V, Ghigo J-M, Beloin C. In vitro activity of gentamicin, Vancomycin or Amikacin combined with EDTA or l-arginine as lock therapy against a wide spectrum of biofilm-forming clinical strains isolated from catheter-related infections. J Antimicrob Chemother. 2015;70(6):1704–12.

Publisher's note