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Assessment of ultrasound irradiation on inactivation of gram negative and positive bacteria isolated from hospital in aqueous solution

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Original Article

Abstract

Microbial contamination of water poses a major threat to public health. With the emergence of microorganisms resistant to multiple antimicrobial agents, there is increased request for promotion of disinfection methods. Since ultrasound wave (US) exhibits antibacterial activities on bacteria, the aim of this study was to evaluate the antimicrobial effect of low frequency (37 kHz) ultrasound on *Pseudomonas aeruginosa* and *Staphylococcus aureus* as a model for gram-negative and gram-positive bacteria, respectively. Sonolysis experiment was carried out in a laboratory-scale batch sonoreactor equipped with plate type transducer at 400 W of acoustic power in the presence and absence of ampicillin as an antibiotic on the both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All of the bacteria were affected by the ultrasound and an increase in percent kill for both bacteria occurred with increasing duration of exposure and intensity of ultrasound. It was found that gram-negative bacteria were more susceptible to the ultrasonic treatment rather than gram-positive bacteria. In addition, the combination of US with an antibiotic (ampicillin) enhanced killing of both bacteria over the use of US alone. The rate of bactericide effect of US wave was increased in samples containing ampicillin. This process was influenced by the chemical and microbiological characteristics of aqueous media. Therefore, with further research about its practicality for treatment of wastewater, it may become a possible substitute process for wastewater disinfection.

KEYWORDS: Ultrasonic Irradiation, Pseudomonas Aeruginosa, Staphylococcus Aureus, Antibiotic, Ultrasonic Frequency

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Introduction

Having many pathogenic, opportunistic microorganisms, and laboratory and pharmaceutical residues, hospital wastewaters are considered as a threat to the public health and environment compared to the municipal wastewater. Therefore, to provide the community health and to prevent the environmental contamination, such wastewaters should be collected in accordance with technical and sanitary regulations, and be properly treated, disinfected and discharged. However, due to the indiscriminate and inappropriate use of different types of disinfectants and the incidence of resistant strains in such centers, not only specific problems have been created (for example nosocomial infections, which is one of the very important health issues[1,2]), but also problems related to the wastewater treatment operations and assurance of an effluent safe and free from microbial agents have been formed. Therefore, disinfection of such types of wastewater and specific look to the new and efficient disinfection methods has always been emphasized.

During recent years, application of ultrasound

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(US) as an efficient and powerful technology has been considered in different disciplines of environmental engineering including water and wastewater disinfection. US results in inactivation of bacteria through the physical, mechanical and chemical mechanisms. During the bubbles explosion, a considerable energy is generated. As a result, the pressures and pressure gradients will cause mechanically weakening and finally destroys bacteria cell wall. Of course, the generated radicals have impact on the biological agents. These free radicals attack to the chemical structure of bacterial cell wall and cause cell wall weakening and destruction.[3] But the most effective way for killing biological agents is the mechanical effects resulted from the bubbles exploding. The microorganisms' cell membrane is decayed because of the pressure intensity produced. These effects include complete destroy or death of microorganisms or bigger organisms and lysis of cell membrane, which results in cell death.[4] On the other hand, application of antibiotics is always considered; not only because of creating antibiotic resistance, but also because of their antimicrobial properties. Therefore, since both US and antibiotics destroy the microbial agents, there is a possibility that their simultaneous application has synergetic effect so that the resistant strains become more sensitive to US and their removal efficiency be increased. Thus, the main objective of the present study was to study the effect of US alone and combined with antibiotic on Pseudomonas aeruginosa (P. aeruginosa) and Staphylococcus aureus (S. aureus), which are among the most important and common infectious agents shown resistance against a wide range of antibiotics.[5,6].

Materials and Methods

It is an experimental-applied study conducted at lab scale at Faculties of Health and Medicine of Kurdistan University of Medical Sciences, Sanandaj, Iran, in 2011. Based on the study aims, the methodology consisted of two parts. In the first part, we handled the P. aeruginosa and S. aureus bacteria obtained from the hospital environment. Therefore, first water samples from different parts of Sanandaj's hospitals (burn, dialysis, operating room, members exchange wards, etc.) were collected and in order to conduct the required tests (temperature, water pH and chlorine content and culturing), they were transferred to the Faculties of Health and Medicine. Then, the loop was cultured on MacConkey and blood agar medium. We used gram strain to identify the microorganisms grown on culture media.[7,8] According to standard methods, oxidase test for gram negative and catalase test for gram-positive bacteria were performed. Differential test was done to detect the Enterobacteriaceae family.[8] After identification of P. aeruginosa and S. aureus, different concentrations [10^2, 10^4, 10^6, and 10^8 colony forming units (CFU)/ml] of both bacteria were prepared and at different time intervals (including 25, 50, 75, and 100 minutes) and in presence of a blank, they were exposed US. The irradiation source of US was a sonoreactor device (Elmasonic P30H, Germany), equipped with a 37 kHz plate adapter with maximum 400 watts power at the laboratory scale. In order to determine the type of antibiotic to be used, the antibiotic susceptibility test was performed using nine antibiotic discs (including amikacin, cotrimoxazole, ampicillin, tetracycline, amoxicillin and vancomycin). Finally, ampicillin was selected and antibiotic doses of 10, 20, and 40 µg were prepared using its 1 g vial. Later, each one was evaluated for its effect on desired bacteria surveillance individually and combined with US for 100 minutes.

In the second part, first strains of P. aeruginosa (PTCC1074) and S. aureus (PTCC1112) were prepared from the Collection Center for Industrial and Infectious Fungi and Bacteria of Iran (Organization of Scientific and Industrial Research of Iran) and cultured according to standard recipes. All stages of the ultrasonic and antibiotics effects on the standard strains were performed according to the first part of study on the isolated strains from hospital environment.

Results and Discussion

In this study, ultrasonic wave's efficiency on degradation of P. aeruginosa and S. aureus and also effect of these waves on effectiveness and improvement of ampicillin on eradication of the resistant strains to this antibiotic has been investigated. Table 1 shows the results of the ultrasonic wave's effect on the strains obtained from environment and on the standard strains of P. aeruginosa and S. aureus over 100 minutes irradiation at different concentrations of each bacteria. As table 1 indicates, the effect of US on reducing the number of P. aeruginosa was more than S. aureus. After 100 minutes, removal of standard strain of P. aeruginosa and S. aureus became 72% and 63% at initial concentration of 10^8 CFU/ml, respectively. These results match well with findings of Villamiel and De Jong.[9] They observed that P. fluorescens (gram negative bacterium) was more sensitive to US compared with S. thermophilus (gram negative bacterium).[9]

Alliger has expressed similar results in the gram-positive bacteria more resistant to US.[10] This difference is due to the characteristics of the bacterial cell wall. Gram-positive bacteria usually contain a dense, strong and thick layer of peptidoglycan compared with gram-negative bacteria so that makes gram-positive bacteria more resistance to US.[11] However, it is believed that the effects of US on the gram-positive and gram-negative bacteria is not yet completely clear.[9] Even some researchers did not observe a significant difference with reference to the effect of US between gram-positive and gram-negative bacteria. In this regard, Scherba et al. studied the effect of US on P. aeruginosa and E. coli (gram-negative bacteria) and S. aureus and B. subtilis (gram positive bacteria) and did not notice any difference between gram positive or gram negative bacteria in terms of the efficiency of ultrasonolysis process.[12] They argued that the main cause of bacteria destroying was the effect of US on the bacterial cytoplasmic membrane; and cell wall is less affected.[12] No significant difference between gram-positive and gram-negative bacteria was observed.

Another notable factor was the effect of process time and bacteria concentration on the removal yield. As table 1 indicates, the bactericide effect of US has increased with time; the concentration of P. aeruginosa and S. aureus from 22 and 19 percent at t = 25 minutes was increased to 69 and 60 percent at t =100 minutes, respectively. These results are consistent with Scherba et al. findings, as they also observed the same trend during their research.[12] However, the linear relationship between contact time and removal percentage of bacteria is logic, and as exposure time of microbial agent with bactericide agent increases, definitely opportunity for the effect of antimicrobial agent increases and removal yield increases. Hence, our results revealed that reducing bacteria concentration enhanced the removal efficiency because reducing number of bacteria results in increasing the possibility of exposure to the ultrasonic wave's irradiation, which in turn, their destroying percent would increase. Therefore, it is noted that reducing the concentration from 10^8 CFU/ml to 10^2 CFU/ml in 100 minutes caused increasing removal efficiency of P. aeruginosa and S. aureus from 69 to 88 percent and from 60 to 77 percent, respectively.

All of the abovementioned experimental procedures were repeated for the standard strains of those bacteria obtained from the media as there is possibility that different bacteria manifest different resistant against ultrasonic irradiation (Table 1). It is noteworthy that no tangible and significant difference was observed between the removal efficiency of standard and media bacteria strains. Although the media strain has shown resistant to some types of antibiotics, this resistant did not have any influence on the efficiency of US in bacteria elimination. Considering that the resistance is a genetic trait and concerns with plasmid,[13] it is concluded that the mechanisms leading to drug resistance or in other words a bacteria resistance, does not cause any change in the sensitivity of bacteria against the US.

Table 1. Removal percentage of P. aeruginosa and S. aureus using ultrasonolysis process under different conditions

Bacteria strain	Bacteria concentration (CFU/ml)	Contact time (min) 0	25	50	75	100
P. aeruginosa (isolated from hospital)	10^2	0	38	61	77	88
	10^4	0	33	55	68	80
	10^6	0	25	44	64	74
	10^8	0	22	35	59	69
P. aeruginosa (standard strain)	10^2	0	41	66	82	92
	10^4	0	35	58	72	86
	10^6	0	28	41	69	81
	10^8	0	25	32	61	72
S. aureus (isolated from hospital)	10^2	0	34	48	63	77
	10^4	0	27	44	53	69
	10^6	0	22	37	49	62
	10^8	0	19	32	43	60
S. aureus (standard strain)	10^2	0	35	52	65	79
	10^4	0	30	47	55	71
	10^6	0	24	41	53	65
	10^8	0	20	34	45	63

CFU: Colony forming units; P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus

Considering the genetic origin of acquiring resistance in bacteria, they manifest different response against the different antibiotics. Therefore, we evaluated the studied bacteria resistance in presence of nine different antibiotics and it was revealed that the studied bacteria were resistant against co-trimoxazole, ampicillin, amoxicillin, and tobramycin and ultimately the effect of ampicillin along with US was studied (Table 2).

The results achieved from this research indicated that the removal efficiency against bacteria is more when combined antibiotic and ultrasonic irradiation is used; this efficiency was less when only the ultrasonolysis process was applied (Figure 1 and 2).

This difference has been contributed to the increase in release of antibiotics from the cell wall liposaccharide layer and its penetration into cell because of the exposure with ultrasonic irradiation. Similar results have been reported by other researchers; for example Johnson et al. studied the combined effect of ultrasonic 70 kHz frequency and the gentamicin antibiotic on reducing E. coli and observed 97 percent reduction in number of bacteria within 2 hours.[14] In another study, Rediske et al. has reported a 2-log increase in mortality of P. aeruginosa by combined process of ultrasonic irradiation and erythromycin antibiotic.[15] It is noteworthy that this efficiency increase cannot be only attributed to antibiotic constituents and in general, ultrasonic irradiation increase bacteria sensitivity to bactericidal agents.[16]

Table 2. Antibiotic resistance of isolated bacteria from hospital

Antibiotic Type	P. aeruginosa	S. aureus
Amoxicillin	Resistance	Resistance
Tobramycin	Resistance	Intermediate
Amikacin	Sensitive	Resistance
Co-trimoxazole	Resistance	Sensitive
Ampicillin	Resistance	Resistance
Tetracycline	Intermediate	Sensitive
Carbenicillin	Resistance	Sensitive
Vancomycin	Resistance	Resistance
Ciprofloxacin	Sensitive	Intermediate

P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus:

Conclusion

In this study, the destruction of P. aeruginosa and S. aureus was assessed using US in the presence of ampicillin. Our results showed that first, US had ability to destroy both bacteria and second, compared to S. aureus, P. aeruginosa bacteria are

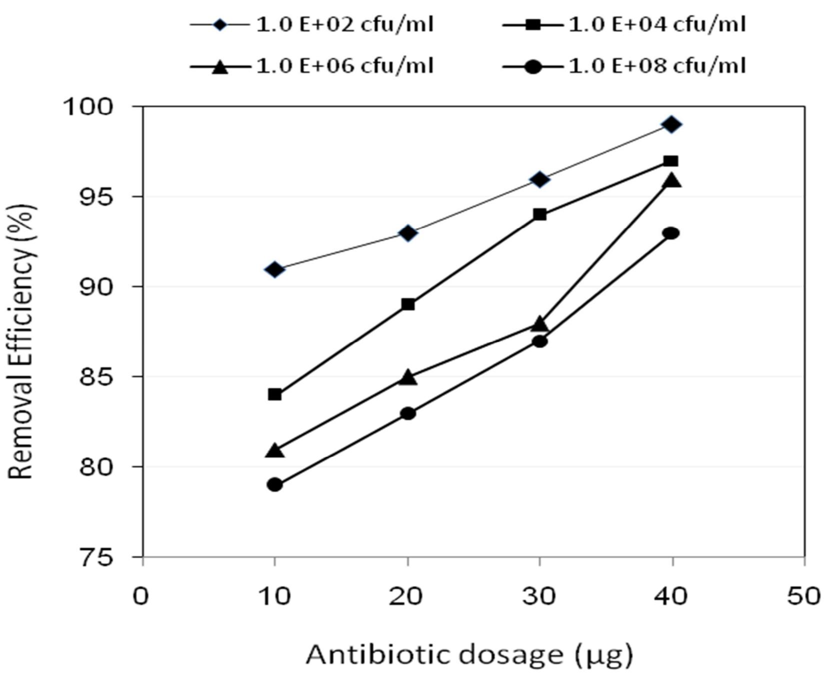

Figure 1. Removal efficiency of isolated P. aeruginosa from hospital under ultrasonic irradiation with ampicillin (t = 100 minutes)

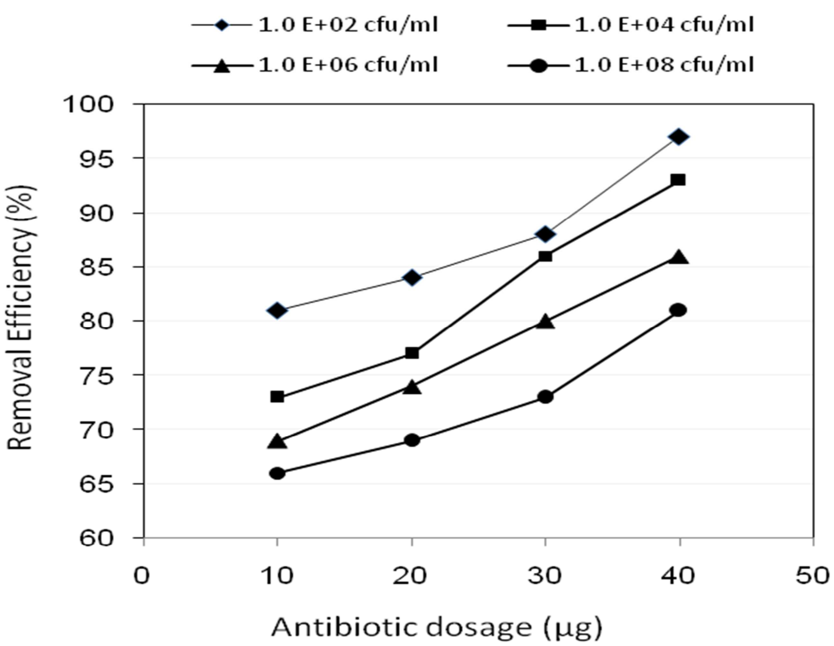

Figure 2. Removal efficiency of isolated S. aureus from hospital under ultrasonic irradiation with ampicillin (t = 100 minutes)

more resistant against US. It was also revealed that the studied bacteria are resistant to ampicillin. Therefore, we studied the effect of ampicillin in presence of US and it was noticed that simultaneous application of US together with antibiotic is more efficient in removing bacteria compared with applying US alone, which can be attributed to the synergistic effect.

The ultrasonolysis experiments on the standard strains of the abovementioned bacteria showed no significant difference in removal efficiency between standard and media strains. Thus, it was revealed that the bacteria resistant had no effect on the destruction efficiency of US.

Conflict of Interests

Authors have no conflict of interests.

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