Original scientific paper
UDC 546.56:543.95

AN OLD/NEW TOOL THAT PREVENTS BACTERIAL SURFACE SURVIVAL

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Abstract

Copper has been used for centuries to disinfect liquids, solids and human tissue. Now it is used as a water purifier, fungicide and as an anti-bacterial agent. It is considered to be safe to humans. The aim of this work was to determine biocide effect of copper on bacterial growth.

Twenty multiresistant strains of each species (*Acinetobacter baumannii, Hafnia alvei, Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*), with strong biofilm formation ability were used. We used two methods to determine copper effect. One was to inoculate broth culture (106 cfu/mL) of each strain on small copper leafs. They were then air-dried for 24 h at room temperature, inoculated onto blood agar plates, and incubated overnight at 37 ^{0}C. The test was repeated with different drying intervals of 1, 6, 12 and 24 h. The second was to prepare same broth culture that was inoculated onto blood agar plates, after what it was covered with copper leafs. Plates were incubated overnight at 37 ^{0}C. When copper leaf was removed plates were incubated additional night.

Copper showed biocidal activity against all 80 bacterial strains. Air-dried leafs of 6, 12 and 24 h, also placed leafs onto agar plates for 24h showed total biocidal activity against all strains. Some of the 1 h air-dried leafs showed little, but most of them showed no growth.

Use of copper with could prevent bacterial adherence and survival both in food processing facilities or hospital environment. In the era of bacterial multiresistance and panresistance we should not surrender the battle, instead we must seek for old/new tools to fight and prevent bacterial survivor in all environments where human health might be affected.

***Key words**: Copper, Bacterial survival, Multiresistance.*

1. Introduction

1.1 Copper is an essential element on Earth

This metal occurs everywhere in the nature. It is present in the earth's crust, oceans, lakes and rivers, as trace element or in large amounts in the form of mine deposits. Every living organism on this planet (plants, animals, humans or even microorganisms), need copper for normal growth and metabolism.

Being present through the process of the civilization development, for more than 10,000 years, it constantly contributed in the social and technological progress. Currently, it is responsible for electricity and water delivery to each of our homes [1].

1.2 Copper as a disinfectant

Copper has been used for centuries to disinfect liquids, solids and human tissue, including the treatment of chest wounds. Today it is used as a water purifier, fungicide and as an anti-bacterial agent [2, 3, and 4]. It is considered to be completely safe to humans [5, 6, 7, and 8].

In March 2008, the US Environmental Protection Agency approved copper to be the first solid material as an antibacterial agent which continuously destroys bacteria [9].

Copper kills pathogenic microbes, which has been shown in various laboratory testing. Its biocide effect is broad-spectrum and with rapid efficacy. Many papers stress that copper has remarkable potent activity in the clinical environment, significantly and continuously reducing bacteria [10, 11, and 12].

1.3 Bacterial survivor on surfaces

It has been reported that both Gram-positive and Gram-negative bacteria are able to survive up to

months on dry inanimate surfaces, with longer persistence under humid and lower-temperature conditions [13, 14, 15, 16, and 17]. Thus, the hospital environment plays a significant role in nosocomial transmission of microorganisms.

Additional problem is the ability of the resistance genes to move from one into other bacteria. Misuse of antibiotics and disinfectants has created a global outbreak of multiresistant microorganisms, even pan-resistant microorganisms, that are resistant towards all groups of antibiotics and many disinfectants. It is clear that we need an urgent plan to fight this resistance problem [18].

But, the real question is - fight this microorganisms with what? New classes of antibiotics? Or new classes of disinfectants? Maybe different therapeutic strategies? Or a straight new non-chemical, non-classical approach towards reversing the resistance problem? Why don't we try to achieve clean hospital surfaces, completely free of bacteria, using the old/new essential Earth elements? Why not using simple and cheap copper strips (Figure 1) to prevent bacterial survivor in our hospitals [19, and 20].

The aim of this work was to determine biocide effect of copper on multiresistance bacterial growth.

Figure 1. Copper strips

2. Materials and Methods

For this purpose, we collected eighty (80) multiresistant strains from various patient infection sites: twenty (20) strains from each species of *Acinetobacter baumannii, Hafnia alvei, Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* as bacteria potent to cause hospital acquired infections.

Strain identification was performed using Microbact 24E (Oxoid UK) and conventional microbiology methods for identification.

Each bacterial strain was tested for antibiotic susceptibility by disc-diffusion method following CSLI [21]. All strains were tested for biofilm formation ability using modified micro titer-plate test [22 and 23].

In the first method we cultured each bacterial strain onto blood agar and incubated over night at 35 ^{0}C. Fresh bacterial culture was suspended into sterile saline (0.89% NaCl) until McFarland 0.5 standard was achieved with approximate cell count density of 1.5 x 10^8 cfu/mL. Bacterial suspension was inoculated onto copper strips. Strips were then air-dried at room temperature. After drying, copper strips were inoculated onto blood agar plates (Columbia agar, Oxoid, UK). Agar plates were incubated overnight at 35 ^{0}C. For each tested bacterial strains the same test was performed with different air-drying intervals of 1, 6, 12 and 24h.

For the second method fresh bacterial culture was suspended into sterile saline (0.89% NaCl) until McFarland 0.5 standard was achieved with approximate cell count density of 1.5 x 108 cfu/mL. Blood agar plates (Columbia agar, Oxoid, UK) were inoculated with prepared suspension from each tested bacterial strain. Blood agar plates were covered with copper strips and then incubated overnight at 35 ^{0}C. After the incubation, copper strips were removed and plates were incubated additional night (Figure 2).

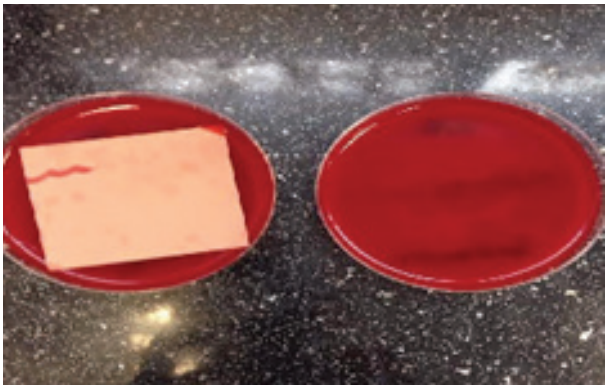

Figure 2. Placing copper strips on Columbia agar after bacterial suspension inoculation

3. Results and Discussion

3.1 Antibiotic susceptibility

Each bacterial strain was tested for antimicrobial susceptibility towards 13 antibiotics using antibiotic discs (Figure 3), following CLSI standard. It was performed on two Mueller Hinton agar plates (Oxoid UK).

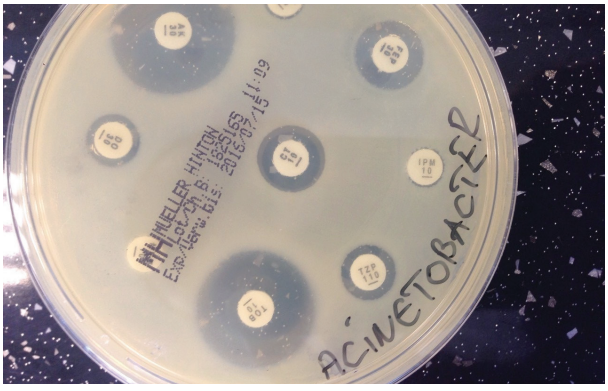

Figure 3. *Acinetobacter baumannii* antibiogram

Every growth inhibition zone was measured in millimeters and interpreted as R - resistant, I - intermediate and S - susceptible, according to the CLSI. The results are shown in Table 1.

Our strains were very resistant to the most of the tested antibiotics. All tested strains are still susceptible only to colistin. According to the results, tested bacterial strains are resistant to most of the tested antibiotics.

3.2 Biofilm formation

Biofilm formation ability for different bacterial strains are shown in Table 2.

All tested strains showed moderate and strong biofilm ability. With high resistance profile and strong biofilm forming potential, bacterial strains are capable of persistence on different surfaces and also may be considered as serious pathogens that can endanger human health.

Table 1. Distribution of antibiotic susceptibility according to the tested bacterial species

Bacterial species / Antibiotic (AB)	AB susceptibility	Number of strains (%)			
		Acinetobacter baumannii	***Pseudomonas aeruginosa***	***Stenotrophomonas maltophilia***	***Hafnia alvei***
Ceftriaxone (30µg)	R	20 (100%)	20 (100%)	20 (100%)	20 (100%)
	I	0	0	0	0
	S	0	0	0	0
Ceftazidime (30µg)	R	20 (100%)	15 (75%)	20 (100%)	15 (75%)
	I	0	3 (15%)	0	2 (10%)
	S	0	2 (10%)	0	3 (15%)
Cefepime (30µg)	R	20 (100%)	9 (45%)	11 (55%)	9 (45%)
	I	0	0	0	1 (5%)
	S	0	11 (55%)	9 (45%)	10 (50%)
Ciprofloxacin (5µg)	R	20 (100%)	6 (30%)	13 (65%)	4 (20%)
	I	0	2 (10%)	0	4 (20%)
	S	0	12 (60%)	7 (35%)	12 (60%)
Doxycycline (30µg)	R	10 (50%)	20 (100%)	20 (100%)	20 (100%)
	I	0	0	0	0
	S	10 (50%)	0	0	0
Gentamicin (10µg)	R	20 (100%)	19 (95%)	20 (100%)	17 (85%)
	I	0	0	0	1(5%)
	S	0	1 (5%)	0	2 (10%)
Amikacin (30µg)	R	20 (100%)	3 (15%)	0	0
	I	0	0	0	0
	S	0	17 (85%)	20 (100%)	20 (100%)
Tobramicin (10µg)	R	20 (100%)	13 (65%)	20 (100%)	5 (25%)
	I	0	0	0	0
	S	0	7 (35%)	0	15 (75%)
Imipenem (10µg)	R	20 (100%)	12 (60%)	4 (20%)	3 (15%)
	I	0	0	3 (15%)	2 (10%)
	S	0	8 (40%)	13 (65%)	15 (75%)
Meropenem (10µg)	R	20 (100%)	13 (65%)	5 (25%)	6 (30%)
	I	0	0	1(5%)	0
	S	0	7 (35%)	14 (70%)	14 (70%)
Trimethoprim-Sulfamethoxazole (1.25/23.75µg)	R	18 (90%)	16 (80%)	10 (50%)	2 (10%)
	I	0	0	0	0
	S	2 (10%)	4 (20%)	10 (50%)	18 (90%)
Piperacillin-Tazobactam (100/10µg)	R	20 (100%)	9 (45%)	8 (40%)	1(5%)
	I	0	1 (5%)	0	1(5%)
	S	0	10 (50%)	12 (60%)	17 (85%)
Colistin (10µg)	R	0	0	0	0
	I	0	0	0	0
	S	20 (100%)	20 (100%)	20 (100%)	20 (100%)

Legend: R = Resistant, I = Intermediate, S = Sensitive.

Table 2. Biofilm forming ability after 24h and 48h of incubation according to the tested bacterial species

Bacterial species / Biofilm forming ability	Number of strains (%)			
	Acinetobacter baumannii	***Pseudomonas aeruginosa***	***Stenotrophomonas maltophilia***	***Hafnia alvei***
Non-adherent	0	0	0	0
Weakly adherent	0	0	0	0
Moderately adherent	3 (15%)	2 (10%)	5 (25%)	7 (35%)
Strongly adherent	17 (85%)	18 (90%)	15 (75%)	13 (65%)

3.3 Copper biocide effect

Copper showed biocidal activity against all 80 bacterial strains of *Acinetobacter baumannii, Hafnia alvei, Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*.

Total biocidal activity against all strains was achieved for all tested strains that were air-dried for 6, 12 and 24h, after inoculation. Three strains of *Stenotrophomonas maltophilia* and two strains of *Pseudomonas aeruginosa* showed growth with negligible levels (taking in consideration of its exposure to a very heavy inoculum), after 1h of air-drying.

Second used method showed total biocidal activity against all tested strains after 24 (Figure 4) and 48 h of incubation.

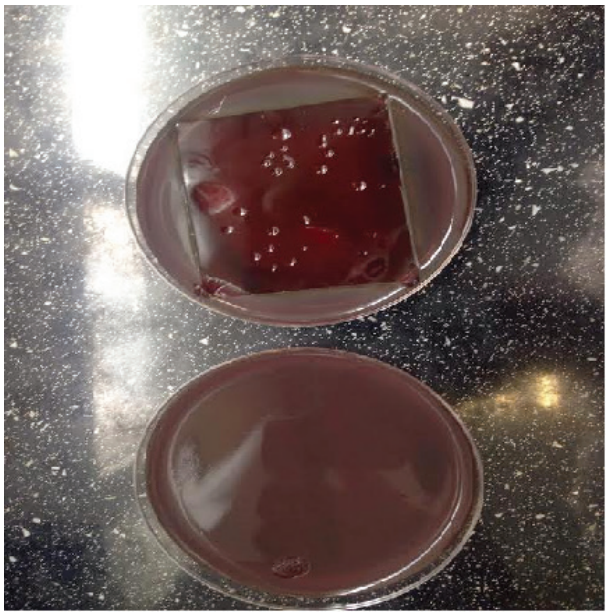

Figure 4. Biocide effect of copper strips after 24h of incubation; positive growth control with no copper strip

After removing the copper strips, there was no bacterial growth even though plates were incubated additional night. The second plate is control plate with no copper strip placed over the inoculated plate. It shows heavy bacterial growth after 48h of incubation.

4. Conclusions

- Results clearly indicate strong antibacterial copper activity.

- Even multiresistant bacteria are highly susceptible to copper.

- Copper is active in cases when contaminated with a very heavy inoculum.

- Bacteria are destroyed already after 1h of copper exposure.

- Copper is an ideal material to be used in hospitals, healthcare settings as well as in the food preparing facilities.

- It is cheap, available and does not wear-out. In cases of re-contamination it remains effective.

- It is safe to use as non-harmful for the environment and humans.

- Copper implementation in hospitals, healthcare settings and food preparing facilities can save over-usage of antibiotics and disinfectants.

- Since it totally disintegrates bacterial biofilms on hospital surfaces, it can prevent resistance epidemics.

- In the era of bacterial multiresistance and panresistance we must not surrender the battle. Instead, we can seek for old/new tools to fight and prevent bacterial survivor in all environments where human health might be affected.

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