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**Effects of silver and zinc oxide nanoparticles incorporated
with chitosan hydrogel beads against some MDR and
biofilm forming bacteria affecting cultured shrimp**

A Thesis

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By

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LIST OF ABBREVIATIONS

<i>A. hydrophila</i>	<i>Aeromonas hydrophila</i>
α- hemolysis	Alpha hemolysis
AMC30µg	Amoxicillin/clavulanic acid
AgNO	Silver nitrite
Ag NPs	Silver Nanoparticles
β hemolysis	Beta hemolysis
cfu	Colony Forming Unit
CTX 30µg	Cefotaxime
CIN Agar	Cefsulodin-Irgasan-Novobiocin Agar
CL30µg	Cephalexin
CN 10µg	Gentamicin
C 30µg	Chloramphenicol
CIP 5µg	Ciprofloxacin
CIT	Citrate utilization
conc.	Concentration
CS	Chitosan
DW	Distilled water.
DO 30µg	Doxycycline
GIT	Gastrointestinal tract
g	Gram
GN card	Gram negative card
hr/s	hour/s.

H₂S	Hydrogen sulphide production
I	Intermediate
ID	Identification number
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-off light Mass Spectrometry
MAR	Multiple Antibiotic Resistance
MDR	Multi Drug Resistance
mg	Milligram
MHA	Mullar Hinton Agar
MHB	Mullar Hinton Broth
ml	Milliliter
min.	Minute(s)
µg	Microgram
µl	Microliter
MSA	Mannitol Salt Agar
MR	Methyl red
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
no.	Number
O.D	Optical Density
ODC	Optical Density cut-off value
P 10 µg	Penicillin
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
ppm	Part per million
R	Resistant

SAM20 µg	Ampicillin/sulbactam
SXT 25µg	Trimthoprim /Sulpha-methoxazole
S	Sensitive
sec.	Second
SEM	Scanning Electron Microscope
SD	standard deviation
<i>S. algae</i>	<i>Shewanella algae</i>
<i>S. putrefaciens</i>	<i>Shewanella putrefaciens</i>
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
TCBS	Thiosulphate citrate bile salt sucrose agar
TCP	Tissue culture plate
TSA	Trypticase soya agar
TSB	Trypticase soya broth
TL15µg	Tylosin
<i>V. alginolyticus</i>	<i>Vibrio alginolyticus</i>
ZnO NPs	Zinc Oxide Nanoparticles

List of Abbreviations of GN card of VITEK system

Well	Test	Mnemonic	Amount/Well
2	Ala-Phe-Ppro-ARYLAMIDASE	APPA	0.0384 mg
3	ADONITOL	ADO	0.1875 mg
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	0.018 mg
5	L-ARABITOL	IARL	0.3 mg
7	D-CELLOBIOSE	dCEL	0.3 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
10	H ₂ S PRODUCTION	H ₂ S	0.0024 mg
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	0.0408 mg
12	Glutamyl Arylamidase pNA	AGLTp	0.0324 mg
13	D-GLUCOSE	dGLU	0.3 mg
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT	0.0228 mg
15	FERMENATATION/GLUCOSE	OFF	0.45 mg
17	BETA-GLUCOSIDASE	BGLU	0.036 mg
18	D-MALTOSE	dMAL	0.3 mg
19	D-MANNITOL	dMAN	0.1845 mg
20	D-MANNOSE	dMNE	0.3 mg
21	BETA-XYLOSIDASE	BXYL	0.0324 mg
22	BETA-Alanine arylamidase pNA	BAlap	0.0174 mg
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg
26	LIPASE	LIP	0.0192 mg
27	PALATINOSE	PLE	0.3 mg

29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
31	UREASE	URE	0.15 mg
32	D-SORBITOL	dSOR	0.1875 mg
33	SACCHAROSE/SUCROSE	SAC	0.3 mg
34	D-TAGATOSE	dTAG	0.3 mg
35	D-TREHALOSE	dTRE	0.3 mg
36	CITRATE (SODIUM)	CIT	0.054 mg
37	MALONATE	MNT	0.15 mg
39	5-KETO-D-GLUCONATE	5KG	0.3 mg
40	L-LACTATE alkalisation	ILATk	0.15 mg
41	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
42	SUCCINATE alkalisation	SUCT	0.15 mg
43	Beta-N-ACYTYL-GALACTOSAMINIDASE	NAGA	0.0306 mg
44	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
45	PHOSPHATASE	PHOS	0.0504 mg
46	Glycine ARYLAMIDASE	GlyA	0.012 mg
47	ORNITHINE DECARBOXYLASE	ODC	0.3 mg
48	LYSINE DECARBOXYLASE	LDC	0.15 mg
52	DECARBOXYLASE BASE	ODEC	N/A
53	L-HISTIDINE assimilation	IHISa	0.0378 mg
56	COUMARATE	CMT	0.126 mg
57	Beta- GLUCORONIDASE	BGUR	0.0378 mg
58	O/129 RESISTANCE (comp.vibrio)	O129R	0.0105 mg

59	GLU-Gly-Arg- ARYLAMIDASE	GGAA	0.0576 mg
61	L-MALATE assimilation	IMLTa	0.042 mg
62	ELLMAN	ELLM	0.03 mg
64	L-LACTATE assimilation	ILATa	0.186 mg

6. SUMMARY

Shrimp covers a major part of the aquaculture industry and as demanding seafood; it comprises 15-20% of total fishery products worldwide. Seafood act as a vehicle for all important species of foodborne pathogens to human and cause serious human disturbances which sometimes leads to death. Therefore, this study aimed to demonstrate the drug-resistant pathogenic bacteria that affected the cultured shrimp, and considered as important human disease factors associated with seafood consumption and try to find an antibacterial alternative to face the worsening problem of antibiotic resistance.

A total number of 250 cultured shrimp samples were collected from different private shrimp farms in Alexandria Governorate, Borg Al-Arab city and Kafr El-Sheikh during the period from May (2018) till June (2019).

A total of 62 bacterial isolates were recovered from cultured shrimp samples including 53 isolates (85.5%) of Gram-negative bacteria and 9 isolates (14.5%) of Gram-positive bacteria. After the biochemical characterization, using conventional phenotypic characteristics and automated VITEK 2 system and then confirmation by MALDI-TOF MS, the results identified the isolated bacteria as following, the most common isolated bacteria among shrimp samples were *V. alginolyticus* constituting 15 isolates (24.2%) followed by *P. aeruginosa* and *A. hydrophila*, 13 isolates (21%) and 12 isolates (19.4%); respectively. Then *Shewanella algae* and *Shewanella putrefaciens* showed moderate to low prevalence constituting 9 isolates (14.5 %) and 4 isolates (6.4%); respectively. On the other hand, *Staphylococcus aureus* was the only isolated Gram-positive bacterial species and it was 9 isolates with a percentage of (14.5%).

On 5% sheep blood agar medium most of the isolated bacteria gave beta hemolysis and some of them gave alpha hemolysis, but few isolates didn't show any hemolytic activity.

Antibiogram sensitivity test and multi-drug resistant bacteria detection showed that some of the tested isolates had a strong resistance to the antibiotic as; *P. aeruginosa* (2 isolates), *Staph. aureus* (2 isolates), *S. algae* (one isolate) *S. putrefaciens* (one isolate) and *A. hydrophila* (one isolate) and some of them showed moderate resistance to the antibiotic as; *S. algae* (2 isolates), *S. putrefaciens* (2 isolates), *V. alginolyticus* (2 isolates), *A. hydrophila* (one isolate), *P. aeruginosa* (one isolate) and *Staph. aureus* (one

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isolate). I tried to find out the mechanism of this resistance by uncovering the biofilm formation ability of these MDR bacteria. Biofilm formation was observed in all tested isolates but with different degrees. I found that; Out of 24 isolates, 16 isolates (66.6%) were strong to moderate biofilm-forming 7 isolates and 9 isolates; respectively. But the other 8 isolates (33.3%) had weak biofilm formation. Also, I found a strong relationship between MDR ability and biofilm formation ability.

I tried to apply alternative anti-bacterial components to counteract antibiotic resistance and biofilm formation. The antibacterial effects of pure chitosan against sensitive, intermediate and strong antibiotic-resistant isolated bacteria were tested, also, I incorporated silver NPS and zinc oxide NPs into chitosan polymer and test the antibacterial effects of them. Blank chitosan beads at 2% concentration showed acceptable inhibitory effects against all the drug-sensitive bacteria and moderately drug-resistant bacteria, but they didn't show any antibacterial effect against the MDR, strong biofilm-forming bacteria. Also, ZnO NPs- CS beads in their low concentration (10- 20 ppm) had a very good antibacterial effect on sensitive and moderately resistant isolates but the antibacterial effect on MDR isolates is low, at high concentrations (50-100 ppm) ZnO NPs- CS beads showed a significant antibacterial effect on all isolated bacteria. Referring to Ag NPs- CS beads, they showed magnificent antibacterial effects on all isolated bacteria at all their tested concentrations.

From the aforementioned results, I found that many antibiotic resistant bacteria were present and were isolated from cultured shrimp. Bacterial diseases affecting shrimp cause significant economic losses in farms, and the presence of antibiotic resistance reduce the efficacy of antibiotic treatment for their infections. Also, there is a strong relationship between MDR ability and biofilm formation ability, as well as increasing antibiotic use and its concentrations to overcome antibiotic resistance and biofilm, makes the condition get worse. So trying to find alternatives to antibiotics has become an urgent necessity. After my experiments on the antibacterial activity of chitosan hydrogel beads and on ZnO NPs and Ag NPs incorporated into chitosan beads, I recommend chitosan as a natural and prophylactic antibacterial alternative to reduce the rate of antibiotic use, I also recommend ZnO NPs (50 ppm) and Ag NPs (10 ppm) which were incorporated into chitosan beads in case of recurrent diseases precedence due to MDR bacteria.