CONTENTS

LIST OF TABLES

LIST OF FIGURES

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	H2S PRODUCTION	H2S	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 alkalinisation	ILATk	0.15 mg
41	ALPHA-GLUCOCSIDASE	AGLU	0.036 mg
42	SUCCINATE alkalinisation	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-HISTIINE 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

This study was carried out to isolate and characterize *A.hydrophila* from fresh, brackish and marine water fishes (different water salinities). Using traditional methods of isolation and biochemical characterization compared to the recent techniques as polymerase chain reaction.

In addition, to compare genetically between the 3 types of *A.hydrophila* isolates by using RAPD PCR. Moreover, check the antimicrobial sensitivity against *A.hydrophila* isolated from fishes live in different water salinities.

A total number of 170 fishes (100 fresh water, 40 brackish water, and 30 marine water fishes) from different farms in Alexandria, Kafr Elsheikh, and Behira governorates were collected from different water salinities. The prevalence of *A.hydrophila* was 47% (38% in fresh water fish, 65% brackish water fish, 53.3% in marine water fish).

A.hydrophila isolates were isolated on R-S media, SMART media, Aeromonas agar media, TCBS media, Nutrient agar, and tryptic soya agar giving the typical colony characters of the bacteria.

A.hydrophila isolates were tested for pathogenicity by inoculation into blood agar and skimmed milk agar 1% for hemolytic and proteolytic activity. All isolates showed β hemolysis and proteolytic activity.

A.hydrophila isolates were inoculated into trypticase soya broth with different NaCl concentration (0.5, 1, 2, 3, 4, 5 and 6%) and turbidity was measured by photometer at wave length 610 nm. The higher NaCl concentration, the lower turbidity was found. Moreover, reduction % of total bacterial count of *A.hydrophila* in relation to different concentration of NaCl in TSB after 10 fold serial dilutions by surface plating technique was calculated.

Hemolysin and aerolysin genes were detected by PCR from the isolates from different sources of fishes (fresh, brackish and marine water fishes).

RAPD PCR was used to differentiate genetically between the bacteria isolated from fresh, brackish and marine water fishes for the first time and we confirmed that there were

genetic variations between the 3 types of isolates upon using 2 RAPD primers. This result explains the reasons why *A.hyrophila* can infect brackish and marine recently as it was known as a disease of fresh water fish mainly.

We tested the tolerance of *A.hydrophila* isolated from different salinities to different NaCl concentrations on broth and media. On broth, bacterial turbidity measured by colorimetric WL and we found that turbidity reduced with higher NaCl concentrations. On media we used total bacterial count and reduction percent to check the effect of NaCl% on total colony count for different dilutions; we found that isolates from marine water fishes can tolerate excess NaCl than fresh and brackish isolates.

A.hydrophila isolates from fresh, brackish and marine fish showed high resistance to Ampicillin (M10), Erythromycin (E15) Nalidixic acid (NA30), and Spectinomycin (SPT10). On the other hand, *A.hydrophila* isolates from fresh, brackish and marine fish showed high sensitivity to Enrofloxacin (EF10), Ofloxacin (OFX5) and Gentamicin (CN10). Polymixin (PB300 u) showed activity against *A.hydrophila* from brackish and marine water fishes only. Doxycycline (DO30) and Nitrofurantoin (F300u) were highly effective against brackish water isolates than others for the field application. Isolation of *A.hydrophila* from different water salinities raises the public health concern and the importance to find suitable methods to control the infection.