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*RESEARCH ARTICLE*

# BACTERIOLOGICAL AND MOLECULAR STUDIES ON BACTERIATRANSMITTED FROM FISHES TO HUMAN.

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## *Abstract*

A total of 1500 samples from 300 apparently healthy fish (280 Tilapia nilotica and 20 Catfish) were collected randomly from some markets and farms at Kafr El-sheikh governorate, Egypt. Also 50 human skin swabs were collected. These samples were collected to isolate and characterize bacteria transmitted from fish to human**.** In this study the prevalence of *Staphylococcus* aureus, *Escherichia* coli, *Salmonella* spp., *Pseudomonas* spp., *Aeromonas* spp. and *Edwardseilla*tarda in Tilapia were 8.2, 21.4, 19, 11.4, 10 and 1.1% respectively, the prevalence of theses pathogens in Catfish were 5, 75, 60, 15, 10 and 0% respectively while its prevalence in human were 8, 24, 20, 12, 0 and 0% respectively. *Escherichia* coli serotype isolates from fish were O153, O1, O125 and O78. *Salmonella* serotype isolates from fish were *Salmonella*inganda, *Salmonella*typhimurium and *Salmonella* Kentucky and *Salmonella*molade. Results showed that all the eight isolates of *Staphylococcus*aureus subjected for determination of *clfA* gene were positive and negative to *Hla* gene, all the five *Salmonella* isolates were positive to *invA* and *sefA* genes and only one from four isolates of *Escherichia* coli was positive to *eaeA* gene and the four isolates were negative to *Hly* gene.



## Introduction:-

Fish diseases are the major problems in fish farm industry. Disease cause economic losses because of high costs of treatment, fish mortality and zoonotic diseases occurred during handling the affected fish **(Magdy*et al*., 2014).**

Fish contain a lot of proteins, vitamins and unsaturated fatty acids. Fish considered the cheapest source of animal proteins.*Staphylococcus* species are the most important food borne opportunistic bacteria which isolated from fish samples, some of *Staphylococcus* species are potential pathogens, and high population of these bacteria indicates the degree of spoilage which might have undergoneand the general quality of fish (**Ali 2014).**

*Escherichia* coli in fish are considered as an indicator of sewage pollution. Most of the *E*.coli is normal inhabitants in the small intestine and they are non-pathogenic, meaning they do not cause disease in the intestine. *E*.coli spread

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outside the intestine cause disease. The pathogenic strains of *E*.coli may cause diarrhea by producing and releasing toxins and cause deaths in fish **(Solimane*t al*., 2010).**

*Salmonella* spp. isolated from freshwater fish such as Catfish. This bacterium has a great risk on human health. *Salmonella* spp. infections can be life-threatening, especially for the very young, the elderly, and for people with immune system problems**(Budiatie*t al*., 2011).**

*Pseudomonas* spp. are widely spread in natural sources of water and associated with septicaemia in aquatic animals. These bacteria are opportunistic pathogens, causing disease when the host exposed to stress **(Magdy*et al*., 2014).**

*Aeromonas*spp. is an opportunistic and zoonotic important bacterium. This bacteria cause diseases in both warm and cold blooded animals as a result of their virulence and pathogenicity. In human cause gastroenteritis, wound infections and chronic diarrhea**( Kamble*et al*., 2012).**

*Edwardseilla*tarda is commonly classified as opportunistic, *E*. tarda is a serious pathogen of fish. This bacterium has a great zoonotic importance. Consumption of infected fish is a cause of gastroenteritis and meningitis **(Lima *et al*., 2008).**

Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on different reasons such as the season, patients' contact with fish, dietary habits and the immune system status of the exposed individual **(Novotny *et al*., 2004).**

With the increasing intensification of aquaculture production, diseases cause problems in the fish farming industry. Although vaccines are being developed and marketed, cannot be used as a universal disease control measure in aquaculture. During the last decades, antibiotics used for fish diseases management, improvement of growth and efficiency of feed conversion **(Denev*et al*., 2009).**

**The aims of this study are to detect, isolate and characterize bacteria transmitted from fish to human.**

## Materials and methods:-

**Sampling:-**

**Fish samples: (Eissa*et al*., 2010 and Hassan *et al*., 2012):-**

A total of 1500 samples (300 skin swab, 300 gills samples, 300 liver samples, 300 spleen samples and 300 kidney samples) from 300 apparently healthy fish (280 Tilapia and 20 Cat fish) were collected randomly from some markets and farms in Kafr El-sheikh governorate (Egypt) during the period from January 2016 to May 2016. 140 Tilapia fish were collected from markets, 140 from farms and 20 Catfish were collected from markets. Fish samples were placed in strong, clean and aseptic bags then packed in column and surrounded with ice bags and brought to laboratory on the day of collection.

**Human samples: (El-olemy*et al*., 2014):-**

A total of 50 human skin swabs were collected with 5 ml saline to avoid dryness of samples.

**Bacteriological examination:-**

**Isolation of bacteria:-**

**Isolation of *Staphylococcus* spp. from fish: (Mus*et al.*, 2014):-**

Skin swab samples were cultured on to nutrient broth with 7% NaCl. The inoculated tubes were incubated at 37°C for 24 hrs.

Nutrient broth samples were streaked on to Baired Parker and Mannitol salt agar plates and incubated overnight at 37 °C.

**Isolation of *E*.coli, *Salmonella* spp., *Pseudomonas* spp., *Aeromonas* spp. and *Edwardseilla* spp. from fish: (Solimane*t al*., 2010), (Hassan *et al.*, 2012) and ( Elissa*et al.*, 2010, El-hady and Samy 2011 and Cagatay and Sen 2014 ):-**

Apiece of liver, spleen, kidney and gills were cultured separately on to MacConkey broth, Tetrathiothianate broth, and Tryptic Soya broth for isolation of ***E*.coli spp.**, ***Salmonella* spp.**, ***Pseudomonas* spp.**, ***Aeromonas* spp., and *Edwardseilla* spp.** Skin swabs were cultured onto Tryptic Soya broth for isolation of ***Pseudomonas* spp.** only. The

inoculated tubes were incubated at incubator. The MacConkey broth samples were streaked on to Eosine Methylene Blue agar for isolation of *E.*coli, the Tetrathiothianates broth samples were streaked on to Salmonella Shigella agar for *salmonella* isolation and the Tryptic Soyas broth samples were streaked on to pseudomonas agar for isolation of ***Pseudomonas* spp**., RimellerShotts agar for isolation of ***Aeromonas* spp. and** *Edwardseilla*media for isolation of ***Edwardseilla* spp.** (selective plating) and incubated overnight at 37 °C**.**

**Isolation of *Staphylococcus* spp., *E*.coli spp., *Salmonella* spp., *Pseudomonas* spp., *Aeromonas*spp. and *Edwardseilla* spp. from human skin swab samples: (El-olemy *et al.*, 2014):-**
**Biochemical identification:-**
The pure colonies of isolates were identified biochemically according to (**Koneman *et al*., 1983, Quinn *et al*., 2002).**

**Serological Identification of *E.coli* serovars according to (Kok*et al.*, 1996) and *Salmonella* serovars according to Kauffman (1974).**

**In-Vitro antibiotic sensitivity of bacteria according to Srivani (2001).**
**Polymerase Chain Reaction (PCR):-**

**Extraction of DNA:-**
According to QIAamp DNA mini kit instructions

**Preparation of PCR Master Mix:-**
According to **Emerald Amp GT PCR mastermix (Takara)** Code No. **RR310A**kit

**Cycling conditions of the primers during cPCR:-**
Temperature and time conditions of the two primers during PCR according to **specific authors** and **Emerald Amp GT PCR mastermix (Takara) kit:-**
They have specific sequence and amplify a specific product as shown in Table (1).

**Table 1:-** Oligonucleotide primers sequencesSource:Metabion (Germany).

| Target MO | Gene | Sequence | Amplified product | Reference |
|---|---|---|---|---|
| *Salmonella* | *InvA* | GTGAAATTATCGCCACGTTCGGGCAA | 284 bp | **Oliveira *et al*., 2003** |
| | | TCATCGCACCGTCAAAGGAACC | | |
| | *sefA* | GCAGCGGTTACTATTGCAGC | 310 bp | **Akbarmehr*et al*., 2010** |
| | | TGTGACAGGGACATTTAGCG | | |
| *S. aureus* | *clfA* | GCAAAATCCAGCACAACAGGAAACGA | 638 bp | **Mason *et al*., 2001** |
| | | CTTGATCTCCAGCCATAATTGGTGG | | |
| | *Hla* | GAAGTCTGGTGAAAACCCTGA | 704 bp | **Fei*et al*., 2011** |
| | | TGAATCCTGTCGCTAATGCC | | |
| *E. coli* | *eaeA* | ATGCTTAGTGCTGGTTTAGG | 248 bp | **Bisi-Johnson *et al*., 2011** |
| | | GCCTTCATCATTTCGCTTTC | | |
| | *Hly* | AACAAGGATAAGCACTGTTCTGGCT | 1177 bp | **Piva*et al*., 2003** |
| | | ACCATATAAGCGGTCATTCCCGTCA | | |

**D- DNA Molecular weight marker.**

The ladder was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.
**E- Agarose gel electrophoreses (Sambrook*et al*., 1989).**

## Results:-

A total of 266 bacterial isolates from fish and 39 bacterial isolates from human were obtained from the examined samples.
*E.*coli was the predominant bacteria isolated from Tilapia with an incidence (21.4%) followed by *Staphylococcus* spp. (19.6%), *Salmonella* spp. (19%), *Pseudomonas* spp. (11.4%), *Aeromonas* spp. (10%) and *Edwardseilla*tarda (1.1%) as shown in (Table 2).

*E*.coli was the predominant bacteria isolated from Catfish (75%) followed by *Salmonella* spp. (60%), *Staphylococcus* spp. (15%), *Pseudomonas* spp. (15%), *Aeromonas* spp. (10%) and there is no *Edwardseilla* spp. were isolated from Catfish as shown in (Table 3).

*E*.coli was the predominant bacteria were isolated from human (24%), (20%) of isolates were from sellers at markets and (28%) from workers at farms. Followed by *Staphylococcus* spp. (22%), (24%) of isolates were from sellers at markets and (20%) from workers at farms. *Salmonella* spp. (20%). (16%) of isolates from sellers at markets and (24%) from workers at farms. *Pseudomonas* spp. (12%). (16%) of isolates from sellers at markets and (8%) from workers at farms. No *Aeromonas* spp. and *Edwardseilla* spp. were isolated from human as shown in (Table 4).

Ten *E*.coli isolates were serotyped, six from Tilapia and four from human, serological identification revealed that five isolates from Tilapia were belonging to (O153, O1, O125 and 2 O78). Three isolates from human belonging to (O153, O26 and O78) as shown in (Table 5).

Ten *Salmonella* isolates were serotyped, six from Tilapia fish and four from human. Serological identification revealed that four isolates from Tilapia belonging to *S*. inganda, *S*. typhimurium, *S*. kentucky and *S*. molade. Two isolates from human were belonging to *S*. typhimurium and *S*. enteritidis as shown in (Table 6).

*E*.coli O153, O1 isolates were resistant to Flumequine but *E*.coli O125 isolates were sensitive to Flumequine. *E*.coli O78, O26 isolates were resistant to Doxycillin but *E*.coliO78 from human was resistant to Ciprofloxacin and Chloramphenicol. It was shown that *S*. kentucky, *S*. molade and *S*. enteritidis were sensitive to Amoxicillin. But *S*. inganda and *S*. typhimurium were resistant to Penicillin (Table 7).

Identification of *clfA* and *Hla* virulence genes of eight *Staphylococcus* aureus isolates and the results revealed that all the isolates contain *clfA* gene but not contain *Hla* gene as shown in (Table 8) and (Figures 1,2).

Identification of *eaeA* and *Hly* virulence genes of four *E*.coli isolates that were serotyped and the results revealed that only one isolate contain *eaeA* gene as shown in (Table 9) and (Figures 3, 4).

Identification of *invA* and *sefA* virulence genes of five *Salmonella* isolates that were serotyped and the results revealed that all the isolates contain both genes as shown in (Table 10) and (Figures 5, 6).

**Table 2:-** Incidence of bacteria isolated from Fish (280 Tilapia and 20 Catfish).

| Type of fish | Number Of Examined Fish | Bacteria | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Staph* spp. | | *E.coli* | | *Salmonella* | | *Pseudomonas* spp. | | *Aeromonas* spp. | | *Edwardseilla* tarda | | Total | |
| | | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % |
| Tilapia & Catfish | 300 | 58 | 19.3 | 75 | 25 | 65 | 21.7 | 35 | 11.7 | 30 | 10 | 3 | 1 | 266 | 88.7 |
| Tilapia | 280 | 55 | 19.6 | 60 | 21.4 | 53 | 19 | 32 | 11.4 | 28 | 10 | 3 | 1.1 | 231 | 82.5 |
| Tilapia from farms | 140 | 23 | 16.4 | 35 | 25 | 23 | 16.4 | 17 | 12.1 | 13 | 9.2 | 0 | 0 | 111 | 79.3 |

**Table 3:-** Incidence of bacteria isolated from Tilapia and Catfish collected from markets.

| Type of fish | Number Of Examined Fish | Bacteria | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Staph*. spp. | | *E.coli* | | *Salmonella* | | *Pseudomonas* spp. | | *Aeromonas* spp. | | *Edwardseilla* tarda | | Total | |
| | | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % |
| Tilapia | 140 | 32 | 22.8 | 25 | 17.8 | 30 | 21.4 | 15 | 10.7 | 15 | 10.7 | 3 | 2.1 | 120 | 85.7 |
| Catfish | 20 | 3 | 15 | 15 | 75 | 12 | 60 | 3 | 15 | 2 | 10 | 0 | 0 | 35 | 175 |

**Table 4:-** Incidence of bacteria isolated from human.

| Sellers and workers | Number of examined | Isolation rate or incidence of bacteria isolated from human | | | | | | Total Number and % Per Fish |
|---|---|---|---|---|---|---|---|---|
| | | *Staph* spp. | *E.coli* | *Salmonella* | *Pseudomonas* spp. | *Aeromonas* spp. | *Edwardseilla* tarda | |

| | sample | ve+ | % | ve+ | % | ve+ | % | ve+ | % | ve+ | % | ve+ | % | ve+ | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| From markets | 25 | 6 | 24 | 5 | 20 | 4 | 16 | 4 | 16 | 0 | 0 | 0 | 0 | 19 | 76 |
| From farms | 25 | 5 | 20 | 7 | 28 | 6 | 24 | 2 | 8 | 0 | 0 | 0 | 0 | 20 | 80 |
| Total | 50 | 11 | 22 | 12 | 24 | 10 | 20 | 6 | 12 | 0 | 0 | 0 | 0 | 39 | 78 |

**Table5:-**Results of serological identification of *E*.coli isolates.

| Serial No. | Identified Bacterium | Serodiagnosis | Strain characterization |
|---|---|---|---|
| 1 | *E*.coli(F) | O153 : H2 | EPEC |
| 2 | *E*.coli(F) | O1 : H7 | EPEC |
| 3 | *E*.coli(F) | O125 : H21 | ETEC |
| 4 | *E*.coli(F) | O78 | EPEC |
| 5 | *E*.coli(F) | O78 | EPEC |
| 6 | *E*.coli(H) | O153 : H2 | EPEC |
| 7 | *E*.coli(H) | O26 : H11 | EHEC |
| 8 | *E*.coli(H) | O78 | EPEC |
| 9,10 not *E*.coli | | | |

(F) *E*.coli isolated from fish. (H) *E*.coli isolated from human.

**Table 6:-** Results of serological identification of *Salmonella* enterica isolates.

| Serial No. | Identified strains | Group | Antigenic structure | |
|---|---|---|---|---|
| | | | O | H |
| 1 | *Salmonella* inganda(F) | C1 | 6,7 | Z10 : 1,5 |
| 2 | *Salmonella* typhimurium(F) | B | 1,4,5,12 | i : 1,2 |
| 3 | *Salmonella* kentucky(F) | C3 | 8,20 | i : Z6 |
| 4 | *Salmonella* molade(F) | C2 | 8,20 | Z10 : Z6 |
| 5 | *Salmonella* typhimurium(H) | B | 1,4,5,12 | i : 1,2 |
| 6 | *Salmonella* enteritidis(H) | D1 | 1,9,12 | g,m : - |
| 7,8,9,10 not *Salmonella* | | | | |

(F) *Salmonella* isolated from fish. (H) *Salmonella* isolated from human.

**Table 7:-** Results of sensitivity test of *E*.coli and *Salmonella* isolates.

| Antimicrobial agents | Diffusion zone break point (mm) | *E*.coli O153(F) | *E*.coli O1(F) | *E*.coli O125(F) | *E*.coli O78(F) | *E*.coli O78(F) | *E*.coli O153(H) | *E*.coli O26(H) | *E*.coli O78(H) | S. inganda (F) | S.typhimurium (F) | S.kentucky (F) | S.molade (F) | S.Typhimurium (H) | S.enteritidis (H) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amoxicillin(AML) | 14≤ | 20(S) | 19(S) | 21(S) | 19(S) | 19(S) | 21(S) | 19(S) | 22(S) | 21(S) | 16(I) | 22(S) | 22(S) | 21(S) | 21(S) |
| Penicillin(P) | 20≤ | 29(S) | 31(S) | 30(S) | 21(I) | 23(I) | 28(S) | 33(S) | 30(S) | 8(R) | 9(R) | 30(S) | 22(I) | 33(S) | 31(S) |
| Ciprofloxacin(CP) | 12≤ | 17(S) | 5(R) | 8(R) | 15(I) | 19(S) | 16(I) | 19(S) | 11(R) | 18(I) | 14(I) | 15(I) | 10(R) | 10(R) | 10(R) |
| Chloramphenicol(C) | 15≤ | 18(S) | 10(R) | 8(R) | 16(I) | 17(I) | 15(I) | 15(I) | 4(R) | 20(S) | 9(R) | 19(S) | 21(S) | 22(S) | 20(S) |
| Erythromycin(E) | 13≤ | 23(S) | 24(S) | 25(S) | 11(R) | 3(R) | 5(R) | 8(R) | 22(S) | 8(R) | 10(R) | 8(R) | 23(S) | 23(S) | 25(S) |
| Neomycin(N) | 12≤ | 19(S) | 18(S) | 17(S) | 13(I) | 19(S) | 18(S) | 19(S) | 19(S) | 7(R) | 15(I) | 12(R) | 22(S) | 15(I) | 20(S) |
| Doxacillin(DO) | 16≤ | 17(I) | 20(S) | 17(I) | 21(S) | 20(S) | 19(S) | 17(I) | 20(S) | 19(S) | 17(I) | 20(S) | 21(S) | 19(S) | 17(I) |
| Flumquine(UB) | 15≤ | 18(S) | 13(I) | 20(S) | 6(R) | 21(S) | 22(S) | 8(R) | 17(S) | 13(I) | 16(S) | 13(I) | 18(S) | 20(S) | 13(I) |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sulphamethoxazol+ trimethoprime(SXT) | 10≤ | 8(R) | 4(R) | 16(S) | 18(S) | 12(I) | 19(S) | 20(S) | 20(S) | 17(S) | 18(S) | 20(S) | 16(S) | 17(S) | 17(S) |

(F)Bacteriaisolated from fish (H) Bacteriaisolated from human

**Table 8:-** Results of molecular identification of *Hla* and *clfA* gene of *Staph*.aureus.

| **Target MO** | **Sample** | **Results** | |
|---|---|---|---|
| | | ***Hla*** | ***clfA*** |
| *S*. aureus | 1 | - | + |
| | 2 | - | + |
| | 3 | - | + |
| | 4 | - | + |
| | 5 | - | + |
| | 6 | - | + |
| | 7 | - | + |
| | 8 | - | + |

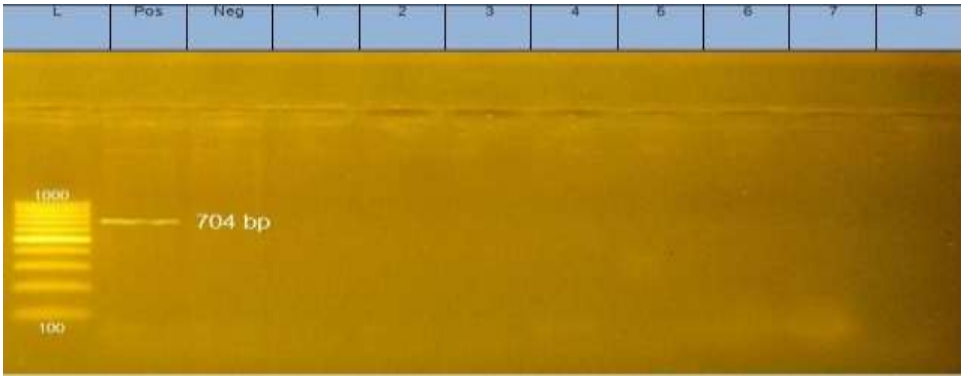


**Fig 1:-**Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control of *Hla* virulence gene of *Staphylococcus*aureus. The size in base pairs (704bP) of PCR product is indicated for the bands.

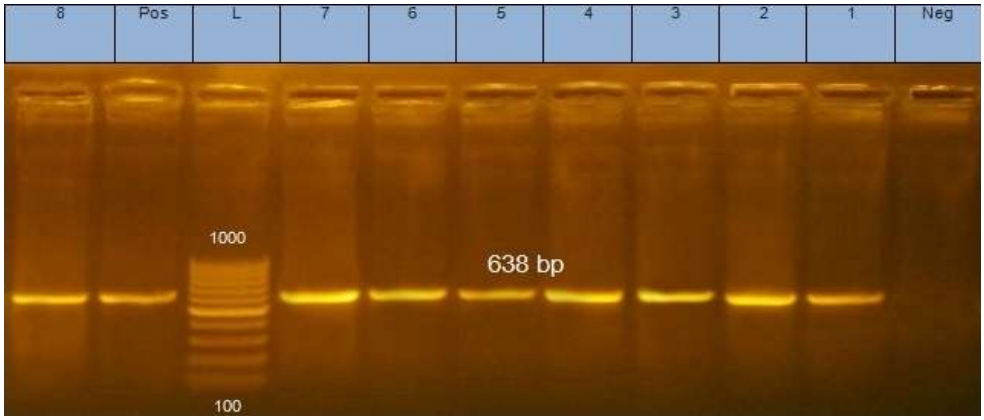


**Fig 2:-**Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control, lane 1, 2, 3, 4, 5, 6, 7, and 8: *clfA* virulence gene of *Staphylococcus*aureus. The size in base pairs (683bP) of PCR product is indicated for the bands.

**Table 9:-** Results of molecular identification of *eaeA* and *Hly*gene of *E*.coli.

| **Target MO** | **Sample** | **Results** | |
|---|---|---|---|
| | | ***eaeA*** | ***Hly*** |
| *E*. coli | 1 | - | - |
| | 2 | + | - |

| | | | |
|---|---|---|---|
| | 3 | - | - |
| | 4 | - | - |

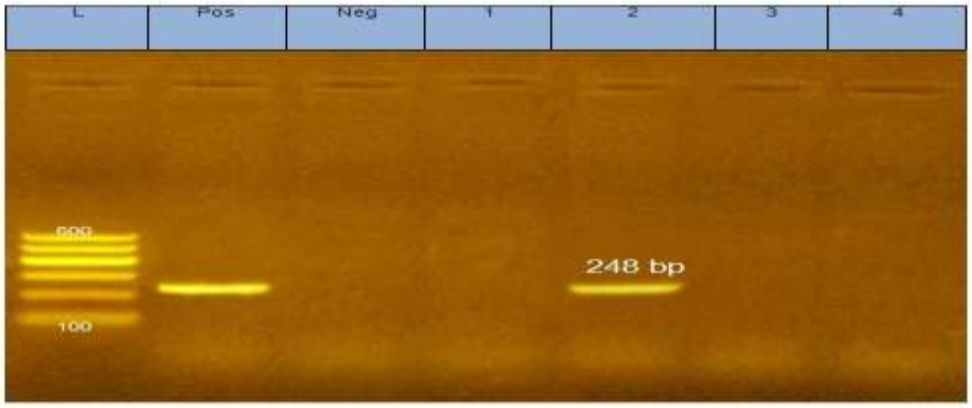


**Fig 3:-**Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control, lane 2: *eaeA* virulence gene of *E*.coli. The size in base pairs (248bP) of PCR product is indicated for the bands.

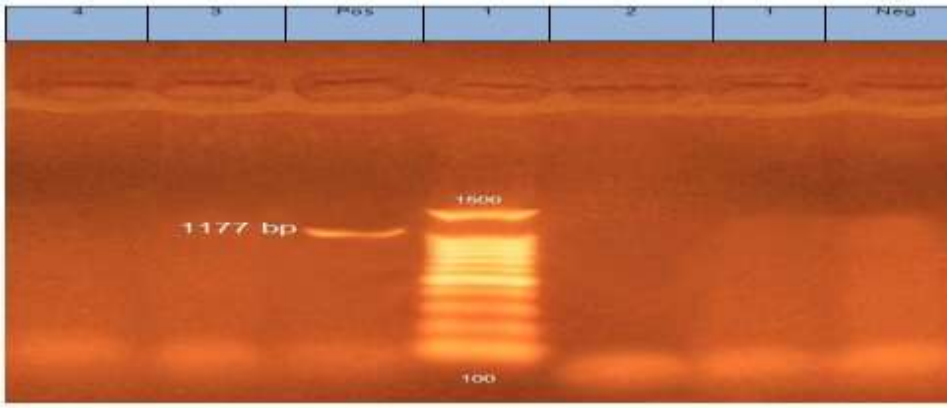


**Fig 4:-**Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control of *Hly* virulence gene of *E*.coli. The size in base pairs (1177bP) of PCR product is indicated for the bands.

**Table 10:-** Results of molecular identification of *invA* and *sefA* gene of *Salmonella*.

| **TargetMO** | **Sample** | **Results** | |
|---|---|---|---|
| | | ***invA*** | ***sefA*** |
| *Salmonella* | 1 | + | + |
| | 2 | + | + |
| | 3 | + | + |
| | 4 | + | + |
| | 5 | + | + |

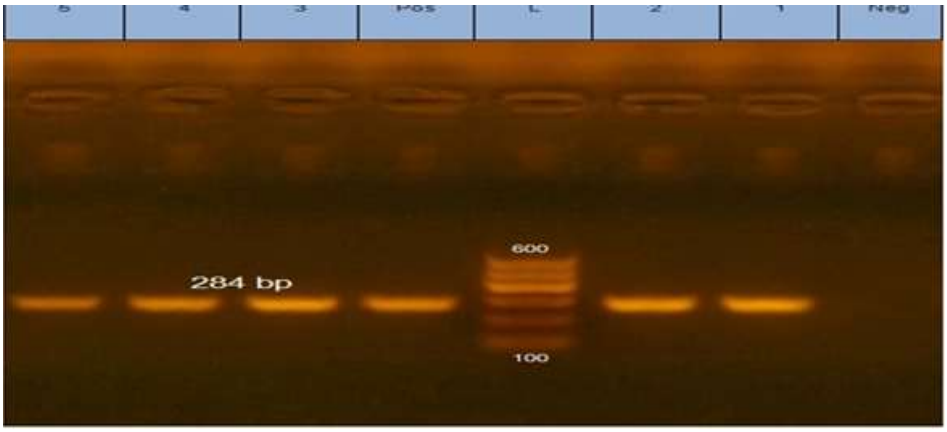


**Fig 5:-**Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control, lane 1, 2, 3, 4 and 5: *invA* virulence gene of *Salmonella.* The size in base pairs (284bP) of PCR product is indicated for the bands.

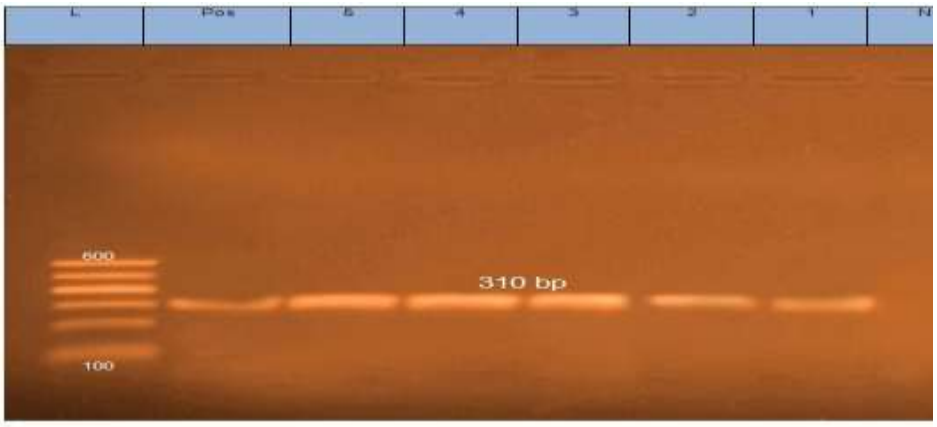


**Fig 6:-**Agrose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Neg: Negative control, lane Pos: Positive control, lane 1,2,3,4 and 5: *sefA* virulence gene of *Salmonella.* The size in base pairs (310bP) of PCR product is indicated for the bands.

## Discussion:-

*Staphylococcus* species are potential pathogens, and high population of these bacteria indicates the degree of spoilage which might have undergone**(Ali 2014).**

In this study, *Staphylococcus*spp. isolated from Tilapia (Oreochromisniloticus) with an incidence of (19.6%) as mentioned at (Table 2).While lower incidences of isolation rate recovered by **El-olemy et al. (2014), Makilla (2014)** and **Mus et al. (2014)** with an incidences 4.5% , 0% and 6% respectively. But **Atwa et al. (2017)** isolated *S.* aureus, *S.* epidermidis and *S.* saprophyticus from skin of Tilapia with incidences 12.5, 23.8 and 31.3% respectively and from liver 15, 12.5 and 16.3% respectively. While *Staphylococcus* spp. isolated from Catfish (Claris lazera) with an incidence of (15%) as mentioned at (Table 3). Our results partial agree with **Toyo et al. (2012)** reporting incidence (13.0%).

On the other hand, *Stphylococcus*spp. isolated from human with incidences of (24%) from sellers in markets and (20%) from workers in farms as mentioned at (Table 4). Higher incidence of *Staphylococcus* spp. recovered by **El-olemy et al. (2014)** reporting incidences (35%) from fish handlers and (37.5%) from house wife's.

*Escherichia* coli in fish are considered as an indicator of sewage pollution. *E.*coli is a bacterium that commonly lives in the intestine of people, animal and fish**Soliman et al.(2010 ).**

*E*.coli isolated from Tilapia (Oreochromisniloticus) with an incidence of (21.4%) as mentioned at (Table 2). Our results partial similar with **Hassan et al. (2012)** and**Saqr et al. (2016)** reporting incidences (27%) and (18.3%) respectively. While higher incidences of *E*.coli were recovered by**Amr et al. (2012), David et al. (2009), Galal et al. (2013) and Gupta et al. (2013)** reporting incidences 50%, 57.1%, 29.34% and 36% respectively. But **Atwa et al. (2017)** isolated *E*.coli from skin, muscle, intestine and liver with incidences 25, 22.5, 25 and 35% respectively. *E*.coli isolated from Catfish (Claris lazera) with an incidence of (75%) as mentioned at (Table 3). Higher incidence of *E*.coli was recovered by **Amr et al. (2012)** reporting incidence (100%). On the other hand, lower incidence of *E*.coli recovered by **Toyo et al. (2015)** with an incidence (23.2%).

On the other hand, *E*.coli isolated from human with incidences of (20%) from sellers in markets and (28%) from workers in farms as mentioned at (Table 4). Our results partially agree with **El-olemy et al. (2014)** reporting incidences (20%) from fish handlers and (37.5%) from house wife's.

*Salmonella* spp. defined as opportunistic and potential pathogenic bacteria of water bodies in warm climate zones. *Salmonella* spp. isolated from freshwater fish such as Catfish. This bacterium has a great risk on human health. **Budiati et al. (2011)**

*Salmonella* spp. isolated from Tilapia (Oreochromisniloticus) with an incidence of (19%) as mentioned at (Table 2). Our results agree with **Hassan et al. (2012)** reporting incidence (21.6%). Higher incidences of *Salmonella* were recovered by **Nwiyi and Onyeabar (2012)** reporting incidences (66.66%) and (50%) from whole body and gills respectively. On the other hand, lower incidences of isolation rate recovered by **El-olemy et al. (2014) and Makilla (2014)** with incidences (11.5%) and (0%) respectively. *Salmonella* spp. isolated from Catfish with an incidence of (60%) as mentioned at (Table 3). While higher incidence of *Salmonella* spp. recovered by **Budiati et al. (2011)** with an incidence (80%). On the other hand, lower incidences of isolation rate recovered by **Amr et al. (2012)** and **Toyo et al. (2015)** with incidences (17.1%) and (7.3%).

*Salmonella* spp. isolated from human with incidences of (16%) from sellers in markets and (24%) from workers in farms as mentioned at (Table 4). But **El-olemy et al. (2014)** isolated it with incidences (35%) from fish handlers and (25%) from house wife's.

*Pseudomonas* spp. is opportunistic pathogens causing disease when the host exposed to stress **Magdy et al. (2014).** *Pseudomonas* spp. isolated from Tilapia (Oreochromis niloticus) with an incidence of (11.4%) as mentioned at (Table 2). But **Atwa et al. (2017)** isolated *P*. aeruginosa from skin, muscle, intestine and liver with incidences 22.5, 20, 17.5 and 25% respectively and *P*. fluorescens from the same organs with incidences 20, 18.8, 15 and 23.8% respectively. On the other hand, *Pseudomonas* spp. isolated from Catfish (Claris lazera) with an incidence of (15%) as mentioned at (Table 3) but **Toyo et al. (2012)** isolated *Pseudomonas* spp. with an incidence (8.7%).

On the other hand, *Pseudomonas* spp. isolated from human with incidences (16%) from sellers in markets and (8%) from workers in farms as mentioned at (Table 4). Also **Sichewo et al. (2013) and Nabih et al. (2016)** isolated it.

*Aeromonas* is an opportunistic and zoonotic important bacterium, Cause diseases in both warm and cold blooded animals as a result of their virulence and pathogenicity **Kamble et al. (2012).**

*Aeromonas* spp. isolated from Tilapia with an incidence (10%) as mentioned at (Table 2). But higher incidences of *Aeromonas* was recovered by **Escarpulli et al. (2003)** reporting incidences of *A*.salmoncidia and *A*.bestiarum (67.5%) and (20.9%) respectively and **Yagananth et al. (2009)** reporting incidence (46.6%). *Aeromonas* spp. isolated from Catfish (Claris lazera) with an incidence (10%) as mentioned at (Table 3). But higher incidence was recovered by **Das et al. (2014)** reporting incidence of *A*.sobria (77.8%).

*Aeromonas* was isolated with an incidence of (0%) from human as mentioned at (Table 4).While **Haenen et al. (2013)** reported that *Aeromonas* transmitted through open wound.

*Edwardseilla* tarda is commonly classified as opportunistic pathogen; *E*. tarda is a serious pathogen of fish. This bacterium has a great zoonotic importance **Lima et al. (2008).**

*Edwardseilla* tarda isolated from Tilapia with an incidence (1.1%) and from Catfish with an incidence (0%) as mentioned at (Table 2, 3). But **Mansoer et al. (2014)** isolated it with an incidence (40%) from Asian catfish.

On the other hand, *Edwardseilla* isolated from human with an incidence of (0%) as mentioned at (Table 4). While **Haenen et al. (2013)** reported that *Edwardseilla* transmitted through open wound.

Ten *E*.coli isolates were serotyped (six from Tilapia and four from human). Serotyping revealed that five isolates from Tilapia were belonging to (O153, O1, O125 and O78) and three isolates from human belonging to (O153, O26 and O78) as mentioned at (Table 5). While **Barbosa et al. (2014)** by serological identification of 49 *E*.coli revealed that the most common serogroups were O125, O126 and O158.

Ten *Salmonella* isolates were serotyped (six from Tilapia and four from human). Serotyping revealed that four isolates from Tilapia belonging to *S*. ingada ,*S*. typhimurium, *S*. kentucky and *S*. molade. Two isolates from human belonging to *S*. typhimurium and *S*. enteritidis as mentioned at (Table 6). But **David et al. (2009)** by serological identification of *Salmonella* revealed that the isolates belonging to *S*. typhimurium, *S*.typhi and *S*. enteritidis.

In this study as mentioned at (Table 7). *E*.coliO153 strain isolated from fish and human, *E*.coli O125 and *E*.coli O78 isolated from fish and *E*.coli O26 were resistant to Doxicyclin. *E*.coli O1 isolates was resistant to Ciprofloxacin, Chloramphenicol, Flumequin and Sulphamethoxazol+trimethoprim. *E*.coli O78 strain isolated from human were resistant to Ciprofloxacin and Chloramphenicol. **Soliman et al. (2010)** reported that *E*.coli isolates were sensitive to Enrofloxacine, Oxanilic acid and Spectinomycine. Erythromycine has least effect. Our results disagree with **Samuel et al. (2011)** who explained that none of *E*.coli shows resistance to Norfloxacine, Sulphamethoxazol+ trimethoprim and Chloramphenicol. *S*. kentucky isolates were resistant to Erythromycine and Neomycin, *S*. molade and *S*. enteritidis isolates were resistant to Ciprofloxacin. *S*. kentucky, *S*. molade and *S*. enteritidis were sensitive to Amoxicillin and Sulphamethoxazol+ trimethoprim. *S*. typhimurium isolates were resistant to Penicillin, Chloramphenicol and Erythromycine. *S*.typhimurium isolated from human was resistant to Ciprofloxacin. But **Nesa et al. (2011)** reported that the isolated *Salmonella* serovars from human were highly sensitive to Ciprofloxacin. While **El-Hadi (2014)** explained the highest antibiotic resistance of *Salmonella* was observed to Tetracycline (90.71%) followed by Amoxicillin (70%) and Amoxicillin+ Clavulanic acid (45%).

As mentioned at (Table 8) all isolates of *Staphylococcus*aureus contain *clfA* gene but not have *Hla* gene, examination of *clfA* gene giving PCR product of (638) bP size and *Hla* gene giving PCR product of (704) bP size . The prevalence of *clfA* gene of *Staphylococcus* aureus was 100% and *Hla* gene was 0%. While **Abdul-Kareem and Husain (2015)** revealed that the prevalence of *hly* gene of *Staphylococcus* aureus in samples was 100%. One isolate of *E*.coli contain *eaeA* gene and none of the isolates contain *Hly* gene. Examination of *eaeA* gene giving PCR product of (248) bP size as shown in (Table 9). The prevalence of *eaeA* gene of *E*.coli was 25%. And this agrees with **Kargar and Hamayoon (2015)** at which only one isolates from seven isolates of *E*.coli O157:H7 contains *eaeA* gene but not has *hlyA* gene. But **Aljanaby and Alfaham (2017)** revealed that the lower prevalence of virulence genes in *E*.coli were (4%) of *eaeA* and *stx1*. As mentioned at (Table 10) all isolates of *salmonella* contain *invA* and *sefA* genes, examination of *invA* gene giving PCR product of (284) bP size and *sefA* gene giving PCR product of (310) bP size. The prevalence of *invA* and *sefA* genes of *Salmonella* was 100%. This agree with **Amalia et al. (2014)** at which PCR product for *invA* gene of *Salmonella* appear at (284) bP size. Moreover, only one sample contains *invA* gene of *S*.typhimurium and five samples contain *sefA* gene of *S*. enteritidis **yadav et al. (2017).**

## Conclusion and Recommendation:-

We can concluded that the most important bacteria causing severe losses in fish are *Staphylococcus* spp., *E*.coli, *Salmonella* spp., *Pseudomonas* spp., *Aeromonas* spp. and *Edwardseilla* spp., these bacteria can transported to human and cause disease. So we recommended the following items:-

1. Adequate cleaning and sanitization of utensils that the fish preserved on it.
2. Effectively training for workers at farms and sellers at markets in hygiene and safety.
3. Application of strict hygienic measures during handling of fish.

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