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# Molecular characterization and antimicrobial effect of some antibiotics on *Aeromonas hydrophila* isolated from different sources

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**ABSTRACT**

The global assessment of the spread of treatment resistance among *Aeromonas hydrophila* identified this bacterium as one of the most opportunistic human diseases. So, the purpose of this study was to evaluate antibiotic resistance of 44 *Aeromonas hydrophila. (A. hydrophila)* isolates previously isolated from different samples representing *African catfish (Clarias gariepinus)*; cow's milk; beef; ground water and diarrheic human stool. In addition, determination of some antimicrobial resistant genes was carried out. The findings showed that all *A. hydrophila* were resistant to ampicillin, then to methicillin, oxacillin, and amoxicillin, as well as to cefotaxime, tetracycline, streptomycin, and co-trimethoprim. At the same time, they were highly sensitive to meropenem followed by norfloxacin, ciprofloxacin; gentamicin; florphenicol and doxycycline. Antimicrobial resistant-$bla_{TEM}$ and $bla_{CTX-M}$ genes were determined in seven out of eight studied strains of 516 bp., and 593 bp., respectively. Meanwhile, $sul_1$and $aad_{A1}$ antimicrobial resistant genes were detected in all eight studied strains giving products of 433 bp. and 484 bp., respectively. Six of *A. hydrophila* strains revealed $tet_{A(A)}$ resistance gene at 576 bp. Therefore, this study concluded that, consumers may have public health concerns if antibiotic resistant *A. hydrophila* strains are found in foods derived from animals (meat, milk), *African catfish*, and groundwater.

## 1. INTRODUCTION

Widespread Gram-negative bacterium called *Aeromonas hydrophila* can cause disease in both warm- and cold-blooded animals (Rasmussen-Ivey *et al.*, 2016; Zhou *et al*., 2019). Typically, these bacterial strains were isolated from a variety of sources, including ground water, fish, meat, dairy products, chlorinated water used for hospital purposes, and human stools (Ugarte-Torres *et al*., 2018).

*A. hydrophila* causes hemorrhagic septicemia in fish and gastroenteritis in humans (Piotrowska and Popowska, 2014).

An important global concern is the rise of antimicrobial resistance in various types of bacteria (Chugh, 2008; Laith and Najiah, 2013; Li and Webster, 2018). *Aeromonas* species' antimicrobial resistance has recently increased as a result of strains isolated from a number of food industry sources as well as clinical isolates showing increased resistance (Alcaide *et al.*, 2010). The distribution of antimicrobial resistance among pathogens transmitted through food has elevated, probably due prolonged medication usage as growth promoters in the lifestyle need for human uses (Kűmmerer, 2009; Adebayo *et al*., 2012; Van Boeckel *et al*., 2015; Deng *et al*., 2016). In addition, water chlorination, antibiotic chemotherapy is the common practice in aquaculture to treat this pathogen leading to their disposing into the environment by run-off water, sedimentation of feces, or uneaten feed pellets that can then be eaten by local fish or invertebrates resulting in the emergence of multidrug-resistant strains of *A. hydrophila* (Andrieu *et al*., 2015; Easwaran *et al*., 2017; Muziasari *et al*., 2017).

Moreover, the widespread use of various groups of beta-lactams; tetracyclines, quinolones, and second-and third-generation cephalosporins for prophylaxis and treatment of *A. hydrophila* in humans; fish farms and animals are considered one of the main causes of the increasing *A. hydrophila* resistance to these antibiotic groups (Saavedra *et al*., 2004; Jacobs and Chenia, 2007; Jun *et al*., 2010; Igbinosa and Okoh, 2012).

Antimicrobial resistance is facilitated by the presence of antimicrobial resistance genes (ARG), and it is established that these genes usually have environmental origins (Lupo *et al*., 2012; Marti *et al*., 2013). In addition to food and animal production farms, hospital effluents have been identified as a source of these ARGs that eventually get transported and transferred by horizontal gene transfer in aquatic environments (Picàò *et al*., 2013). Mobilization of these ARGs by genetic elements and mobile genetic elements such as integrons, transposons and plasmids, means that they can arrive at drinking water supplies, food products and eventually humans (Kűmmerer, 2009; Lupo *et al*., 2012; Marti *et al*., 2013).

In Egypt, antimicrobial resistance is particularly prevalent in *A. hydrophila.* Therefore, this investigation was carried out to assess the antibiotic resistance genes from previously isolated *A. hydrophila* strains collected from different sources at Kaliobia Governorate- Egypt.

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## 2. MATERIAL AND METHODS

*2.1. Samples*

A total of 44 *A. hydrophila* strains were studied. These strains were previously isolated and identified by the authors from 225 random samples of African catfish, cow's milk; beef; ground water and diarrheic human stool. The samples were collected from different fish markets, shops, various localities and hospitals (45 for each) at Kaliobia Governorate, Egypt according to Quinn *et al*. (2002), Nicky (2004) and Markey *et al*. (2013).

*2.2. In-Vitro anti-biotic sensitivity test*

The In-Vitro antimicrobial susceptibility of 44 isolated A. *hydrophila* strains was done against 14 antibiotic types using Kirby–Bauer disk diffusion method according to CLSI (2018) on Mueller-Hinton agar (Oxoid) plates. The following fourteen antimicrobial standardized disks (Oxoid) (their codes and concentrations [µg disc], were tested in antibiograms: {amoxicillin (AMX/25 µg); ampicillin (AMP/10 µg); cefotaxime (CTX/30 µg); ciprofloxacin (CIP/5 µg); co-trimoxazole (COT/25 µg); doxycycline (DO/30 µg); florphenicol (FFC/30 µg); gentamicin (GEN/10 µg); meropenem (MEM\10 µg); methicillin (ME\5 µg); norfloxacin (NOR/10 µg); oxacillin (OX\1 µg); streptomycin (S/10 µg) and tetracycline (TE/30 µg)}.

*2.3. Molecular detection of A. hydrophila strains*

Genotypic detection of five antimicrobial resistant genes, β- lactam (*bla*$_{TEM}$); extended spectrum β-lactam gene (*bla*$_{CTX-M}$); sulphonamide (*sul*$_1$); streptomycin (*aad*$_{A1}$) and tetracycline A *tet* $_{A(A)}$ were carried out. In this regard, eight random *A. hydrophila* strains (two from each *African Catfish*; beef; ground water) and two isolates from both cow's milk and human stool. Briefly, DNA of *A. hydrophila* was extracted following QIAamp® DNA Mini Kit instructions (Qiagen, Germany, GmbH), Emerald Amp GT PCR mastermix (Takara, Japan) with Code No. RR310A and 1.5% agarose gel electrophoreses (Sambrook *et al.,* 1989) using the Primers sequences, target genes, amplicons sizes and cycling conditions (Table 1).

Table 1 Primers sequences, target genes, amplicons sizes and cycling conditions

| Target gene | | Primer sequence (5'-3') | Amplified segment (bp.) | Amplification (35 cycles) Primary Denaturation | Secondary denaturation | Annealing | Extension | Final extension | References |
|---|---|---|---|---|---|---|---|---|---|
| *bla*$_{TEM}$ | F | ATCAGCAATAAACCAGC | 516 bp. | 94˚C, 5 min. | 94˚C, 30 s. | 54˚C, 40 s. | 72˚C, 45 s. | 72˚C, 10 min. | Colom *et al*. (2003) |
| | R | CCCCGAAGAACGTTTTC | | | | | | | |
| *bla* $_{CTX-M}$ | F | ATG TGC AGY ACC AGT AAR GTK ATG GC | 593 bp. | 94˚C, 5 min. | 94˚C, 30 s. | 54˚C, 40 s. | 72˚C, 45 s. | 72˚C, 10 min. | Archambault *et al.* (2006) |
| | R | TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | | | | | | | |
| *sul*$_1$ | F | CGG CGT GGG CTA CCT GAA CG | 433 bp. | 94˚C, 5 min. | 94˚C, 30 s. | 60˚C, 40 s. | 72˚C, 45 s. | 72˚C, 10 min. | Ibekwe *et al*. (2011) |
| | R | GCC GAT CGC GTG AAG TTC CG | | | | | | | |
| *aad*$_{A1}$ | F | TATCAGAGGTAGTTGGCGTCAT | 484 bp. | 94˚C, 5 min. | 94˚C, 30 s. | 54˚C, 40 s. | 72˚C, 45 s. | 72˚C, 10 min. | Randall *et al*. (2004) |
| | R | GTTCCATAGCGTTAAGGTTTCATT | | | | | | | |
| *tet* $_{A(A)}$ | F | GGTTCACTCGAACGACGTCA | 576 bp. | 94˚C, 5 min. | 94˚C, 30 s. | 50˚C, 40 s. | 72˚C, 45 s. | 72˚C, 10 min. | |
| | R | CTGTCCGACAAGTTGCATGA | | | | | | | |

## 3. RESULTS

The findings of in-vitro sensitivity testing are presented in table (2). All *A. hydrophila* showed resistance to ampicillin (100.0%), followed by methicillin and oxacillin (97.7% for each), amoxicillin (93.2%), cefotaxime and tetracycline (88.6% for each), streptomycin (81.8%) and co-trimoxazole (56.8%). On the other hand, they were highly sensitive to meropenem (84.1%) followed by norfloxacin (81.8%), ciprofloxacin (79.6), gentamicin (75.0%); florphenicol (68.2%) and doxycycline (59.1%).

The results of genotyping detection of antimicrobial resistant genes showed that *bla*$_{TEM}$ and *bla* $_{CTX-M}$ genes were determined in 7 out of 8 studied strains giving products of 516 bp. and 593 bp., respectively. *Sul*$_1$ and *aad*$_{A1}$ genes were detected in all 8 screened strains giving products of 433 bp. and 484 bp., respectively. *Tet* $_{A(A)}$ gene was detected in 6 out of 8 strains giving products of 576 bp. (Figures 1-5).

Table 2 In-Vitro anti-microbial Sensitivity test for 44 studied *A. hydrophila*

| Antimicrobial agents | | Disk Concentrations | Sensitive | | Intermediate | | Resistant | | AA |
|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | No. | % | |
| Ampicillin | AM10 | 10 µg | 0 | 0.0 | 0 | 0.0 | 44 | 100.0 | R |
| Methicillin | ME5 | 5 µg | 1 | 2.3 | 0 | 0.0 | 43 | 97.7 | R |
| Oxacillin | OX1 | 1 µg | 0 | 0.0 | 1 | 2.3 | 43 | 97.7 | R |
| Amoxicillin | AMX/25 | 25µg | 0 | 0.0 | 3 | 6.8 | 41 | 93.2 | R |
| Cefotaxime | CTX/30 | 30 µg | 2 | 4.6 | 3 | 6.8 | 39 | 88.6 | R |
| Tetracycline | TE/30 | 30 µg | 1 | 2.3 | 4 | 9.1 | 39 | 88.6 | R |
| Streptomycin | S/10 | 10 µg | 2 | 4.6 | 6 | 13.6 | 36 | 81.8 | R |
| Co- Trimoxazole | COT/25 | (1.25/23.75) µg | 5 | 11.4 | 14 | 31.8 | 25 | 56.8 | R |
| Meropenem | MEM | 10 µg | 37 | 84.1 | 6 | 13.6 | 1 | 2.3 | S |
| Norfloxacin | NOR/10 | 10 µg | 36 | 81.8 | 6 | 13.6 | 2 | 4.6 | S |
| Ciprofloxacin | CIP/5 | 5 µg | 35 | 79.6 | 6 | 13.6 | 3 | 6.8 | S |
| Gentamicin | GEN/10 | 10 µg | 33 | 75.0 | 7 | 15.9 | 4 | 9.1 | S |
| Florphenicol | FFC/30 | 30 µg | 30 | 68.2 | 5 | 11.4 | 9 | 20.4 | S |
| Doxycycline | DO/30 | 30 µg | 26 | 59.1 | 7 | 15.9 | 11 | 25.0 | S |

No.: Number of isolates. AA: Antibiogram activity. %: Percentage in relation to total number of isolates n=44

## 4. DISCUSSION

*Aeromonas hydrophila* has emerged as an entero-pathogen associated with several types of fish and animal infections beside its role in gastrointestinal and extra intestinal infections in humans that often require antimicrobial therapy (Parker and Shaw, 2011; Shah *et al*., 2012) but little is known about the drug resistance among this pathogen in Egypt.

According to estimates of the emergence of drug resistance among *A. hydrophila*, this microorganism has evolved into one of the primary opportunistic human infections (Rey *et al*., 2009; Laith and Najiah, 2013). The recovered results for the antimicrobial sensitivity of 44 *A. hydrophila* isolates declared that all of them were resistant for ampicillin and then they were extremely resistant for methicillin and oxacillin then amoxicillin, cefotaxime, tetracycline, streptomycin and co-trimoxazole. The indiscriminate use of antibiotics in animals and medicine is blamed for the rise in

the occurrence of different antimicrobial resistant bacteria (Del Castillo *et al*., 2013; Dobiasova *et al*., 2014; Cabello *et al*., 2016). The resistance of *Aeromonas* species to β-lactam antimicrobials has most likely increased due to the presence of β-lactamases genes (Ndi and Barton, 2011).

For *A. hydrophila* recovered from fish, milk, meat, water, and stool samples, nearly comparable results were obtained by Subashkumar *et al*. (2006), Ramalivhana *et al.* (2009), Igbinosa and Okoh (2012), Laith and Najiah (2013), Didugu *et al*. (2016), Stratev and Odeyemi (2016), Abd El Tawab *et al*. (2017) and Elbehiry *et al*. (2019). Moreover, the studied *A. hydrophila* isolates were very sensitive to meropenem, norfloxacin, ciprofloxacin, gentamycin, florphenicol and doxycycline. These results agreed with those of Igbinosa and Okoh (2012), Laith and Najiah (2013), Didugu *et al*. (2016), Stratev and Odeyemi (2016), Abd El Tawab *et al*. (2017) and Elbehiry *et al*. (2019).

The present study confirmed that phenotypic double antimicrobial resistances are universal between all isolated A. *hydrophila* strains. These findings are of great importance because the antimicrobials under study, primarily β-lactam antimicrobials, are still widely regarded as the best treatments for bacterial infections in fish, animals, and humans. However, their efficacy has significantly declined due to the production of β-lactamases and other resistant genes by resistant bacterial strains.

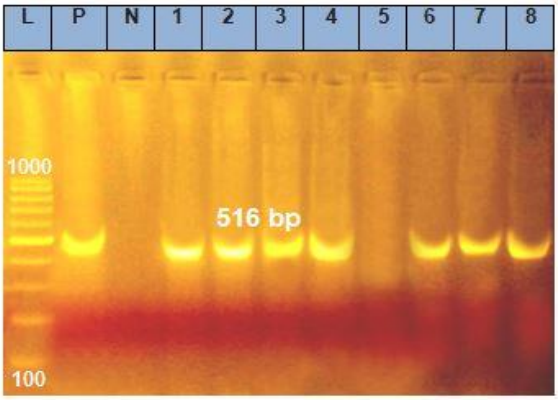


Fig. 1 Agarose gel electrophoresis of β- lactam resistance (*bla*TEM) gene of *A. hydrophila* isolates from different sources. Lane (L): 100-1000 bp DNA Ladder, Lane (P).: Positive control (*A. hydrophila* form Ahri. at 516 bp.), Lane (N): Negative control (*S. aureus* ATCC25923), Lane 1 – 4 and 6-8: Positive *A. hydrophila* at 516 bp. (1and 2 African Catfish; 3 and 4 beef; 6 and 7 ground water and 8 human stool), Lane 5: Negative *A. hydrophila* at 516 bp. (from cow's milk).

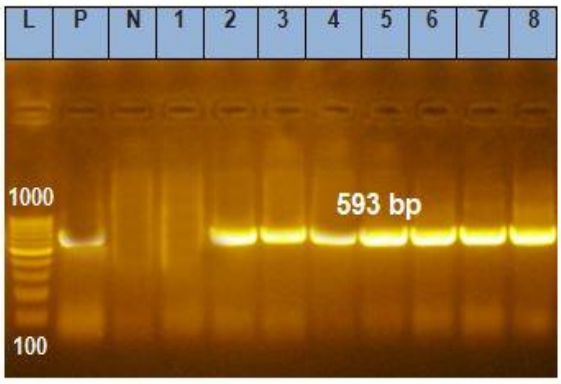


Fig. 2 Agarose gel electrophoresis of Extended spectrum β-lactam resistance (*bla*CTX-M) gene of *A. hydrophila* isolates from different sources. Lane (L): 100-1000 bp DNA Ladder, Lane (P): Positive control (*A. hydrophila* form Ahri. at 593 bp.), Lane (N).: Negative control (*S. aureus* ATCC25923), Lane 1: Negative *A. hydrophila* at 593 bp. (from African catfish), Lane 2 – 8: Positive *A. hydrophila* at 593 bp. (2 African catfish; 3 and 4 beef; 5 cow's milk; 6 and 7 ground water and 8 human stool).

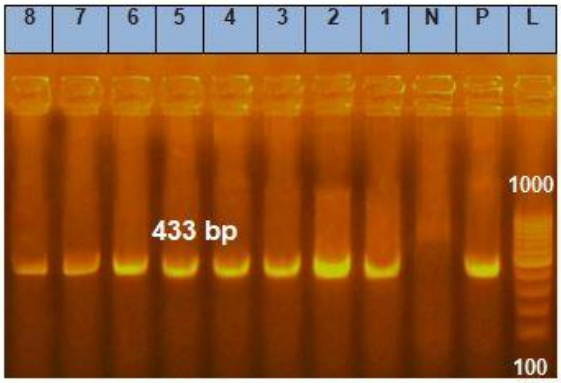


Fig. 3 Agarose gel electrophoresis of β-lactam resistance ($sul_1$) gene of *A. hydrophila* isolates from different sources. Lane (L): 100-1000 bp DNA Ladder, Lane (P): Positive control (*A. hydrophila* form Ahri. at 433 bp.), Lane (N).: Negative control (*S. aureus* ATCC25923), Lane (1 – 8): Positive *A. hydrophila* at 433 bp. (1 and 2 African Catfish; 3 and 4 beef; 5 cow's milk; 6 and 7 ground water and 8 human stool).

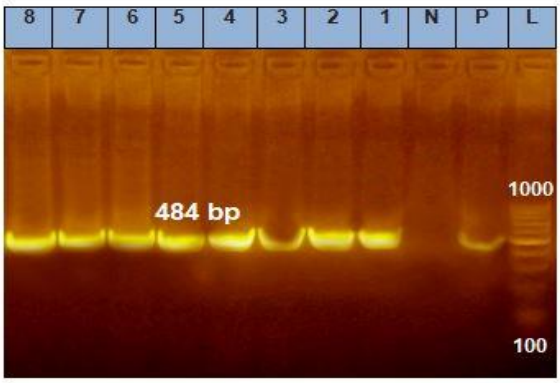


Fig. 4 Agarose gel electrophoresis of β-lactam resistance ($aad_{A1}$) gene of *A. hydrophila* isolates from different sources. Lane (L): 100-1000 bp DNA Ladder, Lane (P): Positive control (*A. hydrophila* form Ahri. at 484 bp.), Lane (N).: Negative control (*S. aureus* ATCC25923), Lane (1 – 8): Positive *A. hydrophila* at 484 bp. (1 and 2 African Catfish; 3 and 4 beef; 5 cow's milk; 6 and 7 ground water and 8 human stool).

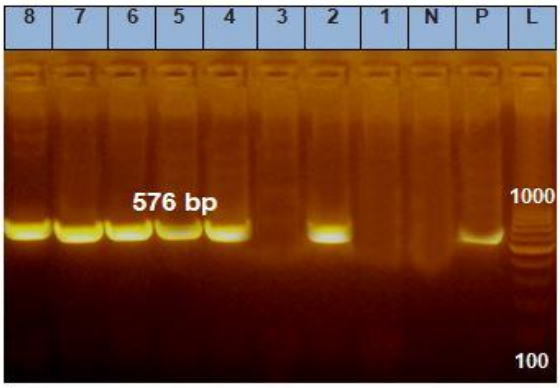


Fig. 5 Agarose gel electrophoresis of β- lactam resistance A ($tet_{A(A)}$) gene of *A. hydrophila* isolates from different sources. Lane (L): 100-1000 bp DNA Ladder, Lane (P): Positive control (*A. hydrophila* form Ahri. at 576 bp.), Lane (N): Negative control (*S. aureus* ATCC25923), Lane 2,4 – 8: Positive *A. hydrophila* at 576 bp. (2 African Catfish; 4 beef; 5 cow's milk; 6 and 7 ground water and 8 human stool), Lane 1 and 3: Negative *A. hydrophila* at 576 bp. (from African Catfish & beef).

So, the present study was directed for recognizing five antimicrobial resistant genes ($bla_{TEM;}$ $bla_{CTX-M;}$ $sul_{1;}$ $aad_{A1}$and $tet_{A(A)}$) in eight random *A. hydrophila* strains. The results revealed that seven out of eight *A. hydrophila* strains have blaTEM and $bla_{CTX-M}$ antimicrobial resistance genes at 516 bp. & 593 bp., respectively. These results in harmony with those were obtained by Awan *et al*. (2009), Tayler *et al*. (2010), Ghenghesh *et al*. (2013), Okolie

(2015), Stratev and Odeyemi (2016), Abd El Tawab *et al*. (2017) and Fauzi *et al*. (2021). Meanwhile, it disagreed with Ndi and Barton (2011), who were unable to identify this pathogenic lactam resistance gene in *A. hydrophila* strains.

Numerous investigations have noted the existence of the β-lactamase gene in *Aeromonas species*, particularly *A. hydrophila*, which is first mediated by β-lactamases, hydrolyzing the β-lactam ring, and inactivating the antibiotic. Although several other β- lactamases encoding genes have been reported, the most prevalent ones in *Aeromonas* include *bla(TEM*), *bla(SHV), bla( OXA-1*), *bla (CMY)*, and *bla(CTX-M*) (Chen *et al*., 2012; Ghenghesh *et al*., 2013) and when using some selective culture media to isolate Aeromonas species from contaminated materials, ampicillin is a good addition (Awan *et al.*, 2009; Daood, 2012). Furthermore, the existence of these genes may result from secondary resistance mechanisms such as β-lactamases or antimicrobial efflux pumps, acquired β-lactams resistance from the expression of chromosomal lactamases, or an increase in intrinsic β-lactam resistance, which is improved by an active efflux mechanism or collaboration out of external membrane impermeability (Tayler *et al*., 2010). Additionally, it is hypothesized that this is due to a high inherent β- lactam resistance level, which is boosted by an active efflux mechanism, collaboration through external membrane impermeability, or secondary resistance mechanisms called beta lactamases or antibiotic efflux pumps (Janda and Abbott, 2010; Tayler *et al*., 2010; Fauzi *et al*.,2021). In the current study, the identification of the sul1gene in all eight *A. hydrophila* strains that produced 433 bp- amplified products was associated with an increase in trimethoprim and sulphamethoxazole resistance. The same outcomes were attained by Ndi and Barton (2011), Igbinosa and Okoh (2012), kore *et al*. (2014), Okolie (2015) and Abd El Tawab *et al*. (2017). Moreover, the $aad_{A1}$ gene was detected in all eight studied strains giving products of 484 bp. which correlated with the phenotypic resistance to streptomycin. This result came in accordance with Verner-Jeffreys *et al*. (2009), Ndi and Barton (2011), Okolie (2015), Abd El Tawab *et al*. (2017) and Fauzi *et al*. (2021).

There were previous investigations into the genetics of tetracycline resistance in Aeromonas species (Schmidt *et al*., 2001). *Aeromonas* species have been classified into five classes of genetically distinctive tetracycline resistance determinants (*tet* A to *tet* E), with *tet*A and *tet*E being the most common (Balassiano *et al*., 2007). In the present study, the *tet* A(A) gene was amplified in only six strains of *A. hydrophila*, and yielded products of 576 bp. The same results were recorded by Verner-Jeffreys et al. (2009), Ndi and Barton (2011), Abd El Tawab *et al.* (2017) and Fauzi *et al*. (2021), though disagreed with Igbinosa and Okoh (2012), who failed to determine *tet* resistant gene in these strains.

## 5. CONCLUSION

Finally, this study found that antimicrobial resistance of *A. hydrophila* isolates in humans; ground water and foods of animal origin should be continuously monitored to avoid public health hazards. The presence of antimicrobial resistant *A. hydrophila* strains in fish, milk, and ground water could be a public health concern for consumers

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