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

Manar E. El Khiaate1, Ashraf A. Abd El Tawab1, Ahmed A. A. Maarouf2, Heba A. N. Afify1

2Department of Microbiology, Animal Health Research Institute, Benha branch

ARTICLE INFO

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 genes were determined in seven out of eight studied strains of 516 bp., and 593 bp., respectively. Meanwhile, sul1 and aadA6 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 M 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 are 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 (Kümmerer, 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; Igbinoso 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 (Picioa 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 (Kümmerer, 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.

* Correspondence to: hebaabdulrezik192@gmail.com
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 Kalobia 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 (blaTEM); extended spectrum β-lactam gene (blaCTXM); sulphonamide (sul); streptomycin (aadA) and tetracycline A tetM 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, ampiclons sizes and cycling conditions (Table 1).

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 blaTEM and bla CTXM genes were determined in 7 out of 8 studied strains giving products of 516 bp. and 593 bp., respectively. SulI and aadA1 genes were detected in all 8 screened strains giving products of 143 bp., respectively. TetM gene was detected in 6 out of 8 strains giving products of 576 bp. (Figures 1-5).

4. DISCUSSION
Aeromona hydrophila has emerged as an enteroto-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.
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-lactamasases 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), Ramalihvama et al. (2009), Igbinsosa 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 Igbinsosa 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-lactamasases and other resistant genes by resistant bacterial strains.

![Agarose gel electrophoresis of β-lactam resistance (blaTEM) gene of A. hydrophila isolates from different sources.](image1)

![Agarose gel electrophoresis of Extended spectrum β-lactam resistance (blaCTX-M) gene of A. hydrophila isolates from different sources.](image2)

So, the present study was directed for recognizing five antimicrobial resistant genes (blaTEM, blaCTX-M, sul1, aadA and tet (M)) in eight random A. hydrophila strains. The results revealed that seven out of eight A. hydrophila strains have blaTEM and blaCTX-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...


