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Molecular Characterization of Antimicrobial Resistance in *Edwardsiella Tarda* Isolated from Diseased Tilapia Fish in Egypt

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Edwardsiella tarda (E. tarda) is a zoonotic bacterium that causes Edwardsiellosis, characterized by extensive mortality in fish leading to high economic losses. Little information is available on the molecular characterization of antimicrobial resistance in *E. tarda*. Therefore, the objective of this research was to depict, at the molecular level, *E. tarda's* antimicrobial resistance isolated from tilapia fish in Egypt, which was identified by both biochemical tests (Microbact™) and polymerase chain reaction (PCR) using species-specific primers targeting the *gyrase B1 (gyrB1) gene*, a taxonomic marker for *E. tarda*, with a size of 415 bp. Twenty-four *E. tarda* isolates were isolated from 100 diseased fish samples randomly collected from 10 private fish farms (ten fish each) in the Kafr El-Sheikh governorate, Egypt. The susceptibility of all isolates was determined against 10 antimicrobial agents (amoxicillin (AMX), ampicillin (AMP), ciprofloxacin (CIP), tetracycline (TE), cefotaxime (CTX), streptomycin (S), erythromycin (E), norfloxacin (NOR), amoxicillin/clavulanic acid (AMC), and sulbactam/ampicillin (SAM). Most of these isolates demonstrate multidrug-resistant phenotypes, as the complete resistance was against erythromycin (100%), followed by the highest resistance against tetracycline and amoxicillin (83.3%). β-Lactamase-encoding genes have been detected as follows: bla_{TEM} and bla_{SHV} in 85.7% and 14.2% of isolates, respectively. While bla_{OXA} and bla_{CTX-M} were not detected, tetracycline resistance genes were identified as follows: *tetA* in 57.1% and *tetB* in 42.8%, but the erythromycin resistance gene (*ermB*) was not detected. This study demonstrated that fish are potential sources of multidrug-resistant *E. tarda.*

Keywords: *Edwardsiella tarda* (*E. tarda*), *gyrB1*, Antimicrobial resistance genes, Fish; Microbact™.

Introduction

Edwardsiellosis is a bacterium that causes a devastating sickness in fish. Associated with fish handling by nets, overpopulation, poor water quality, high organic matter, along with elevated organic matters, as well as water temperature, *E. tarda* generates substantial mortality (40-70 percent) in cultured freshwater fish [1]. Many types of fish are now seriously infected [2], such as catfish with skin lesions with emphysematous disease [3]. *E. tarda* has

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a bad effect on growth, fecundity, and productivity [4].

Among the most serious hazards to aquaculture is *Edwardsiella*, as *E. ictaluri, E. tarda, E. piscicida, E. hoshinae, & E. anguillarum* are the most common species[5]. *Edwardsiella tarda* is the first member of the genus *Edwardsiella,* which is a member of the *Enterobacteriaceae*, causing intracellular infection [6] and high antibiotic resistance, which in turn transfers to other human or animal pathogens [7]. The bacterium is facultatively anaerobic, and it's the causative agent of Edwardsiellosis. *E. tarda* is a normal inhabitant of the fish intestine, & with stress factors such as high temperatures and poor environmental conditions, it becomes virulent. Because *E. tarda* is zoonotic, it increases the risk of human infection, posing health risks as a result [8].

Diagnosing *E. tarda* in tilapia fish often requires conventional polymerase chain reaction due to its speed and reliability [9], by detecting the *gyrB1* gene. *GyrB* has been used to identify, classify, and discriminate a diverse group of bacteria & is also more precise than the 16S *rRNA* gene in distinguishing between *Edwardsiella* spp. and other closely related *Enterobacteriaceae* members [10, 11], as the degree of similarity between *Edwardsiella* spp. in the *gyrB* gene is less than that in the 16S *rRNA* gene.

Antibiotic-resistant pathogens are a major contemporary health and aquaculture concern [12], and animals in the world. Antibiotics are being misused, resulting in antibiotic-resistant bacteria [13, 14]. Antibiotic-resistant bacteria occur through horizontal gene transfer or mutation [15]. Human infection from aquatic bacteria with resistant bacteria resulted in human health danger [13]. *E. tarda* has developed a resistance to nearly all available antibiotics [7], so these antibiotic-resistant isolates make the treatment of Edwardsiellosis difficult. Antibiotic resistance genes have been discovered on the chromosomes of bacteria [16]. According to a previous study, *E. tarda* possessed hereditary antimicrobial resistance genes [17]. To prevent the emergence of public-health-related strains of antibiotic resistance, molecularly identified antibiotic-resistance genes that are inherited should be frequently assessed [18]. Our work highlighted antibiotic resistance by using new antibiograms that may have a higher chance of sensitivity than older antibiograms used in our local area. The purpose of this research was to determine the genetic mechanisms responsible for *E. tarda*'s resistance to antibiotics in Kafr El-Sheikh, Egypt's tilapia fish (*Oreochromis niloticus*).

Material and Methods

Fish sampling

Randomly, 100 diseased tilapia fish (*O. niloticus*) were collected between June and August of 2021 from the Kafr El-Sheikh governorate farms. Samples of diseased fish were taken then transferred alive and kept in sterilized containers to the microbiology lab, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, under aseptic conditions without delay to conduct bacteriological, clinical, and postmortem tests.

All fish handling was done in accordance with the standards for maintaining and making use of animals for scientific purposes that were formulated by the Ethics Committee of the Faculty of Veterinary Medicine at Kafrelsheikh University in Egypt. These guidelines were followed for all fish handling.

Clinical & postmortem examination

External and internal pathological abnormalities were investigated clinically in the fish for the detection of abnormalities associated with

Edwardsiellosis following the standard protocols [19]. Fish were examined for post-mortem signs as previously described [20].

Isolation of E. tarda and identification

Aseptically, after skin disinfection with 70% ethyl alcohol, bacterial isolate was done using samples taken from the liver, skin lesions, spleen , gills, and kidneys of infected fish then inoculated 1st in tryptic soy broth at 32°C for 8–12 hrs, then in MacConkey broth at 32°C for 12–24 hrs. Then cultured on Salmonella-Shigella agar followed by incubation from one to two days at thirty degrees Celsius, as previously described [21]. All isolates were identified using culture characteristics, Gram staining, motility testing, and biochemical identification by microbact (oxoid) for oxidase-negative isolates. Because *E. piscicida* and *E. tarda* have phenotypic similarities, PCR was utilized to validate the identity of the retrieved isolates using species-specific primers targeting the *gyrB1* conserved gene of *Edwardsiella tarda,* as previously stated [22] (Table 1).

E. tarda isolates' antimicrobial susceptibility

The antibiotic sensitivity phenotypes of bacterial isolates were estimated using the Kirby-Bauer disc diffusion assay on Muller Hinton agar plates (Oxoid, UK) & the plates were underwent incubation at 37°C for one day consistent with the Clinical and Laboratory Standards Institute (CLSI) [23] interpretive criteria. The following antimicrobial agents were involved: amoxicillin, 10 µg; ciprofloxacin, 5 µg; ampicillin, 10 µg; tetracycline, 30 µg; streptomycin (S), 10 µg; cefotaxime (CTX), 30 µg; erythromycin (E), 15 µg; norfloxacin (NOR), 5 µg; amoxicillin/Clavulanic acid (AMC); and sulbactam/ampicillin (SAM), 30 µg (Oxoid, UK). Fish farms in Egypt make extensive use of the discovered antibacterial compounds. *Escherichia coli* ATCC 25922 were employed as a standard for quality assurance. Clinical Laboratory Standards Institute criteria were used for the study's analysis & measurement of the inhibitory zone's diameter. [23].

DNA extraction & molecular characterization of antimicrobial resistant genes of E. tarda

Following the manufacturer's instructions, all of the isolates had their DNA extracted using the QIAamp DNA micro kit (Cat. No. 51304, Thermo Fisher Scientific, Qiagen). Multiplex PCR assays were used to detect *ermB,* and *(tetA* and *tetB),* which are responsible for resistance to erythromycin and tetracycline antibiotics in *E. tarda* strains, respectively. Duplex PCR assays were used to detect (bla_{TEM}, and $bla_{\text{OXA-1}}$), (bla_{SHV}, and $bla_{\text{CTX-M}}$), which are responsible for resistance to β-Lactamase inhibition antibiotics in *E. tarda* strains, respectively. The primer sequences, target genes, amplicon sizes of the used genes, and relevant references are summarized in Table 1 [22, 24-28].

The positive controls used for PCR techniques are local strains containing the target genes, while we used deionized water as a negative control.

PCR experiments were done on a T3 thermal cycler with the same circumstances. The PCR cycle involved initial denaturation at 94˚C for 5 min, 35 cycles of denaturation for 30 sec. at 94˚C, primer annealing (50˚C for 40 sec. to *E. tarda gyr*B, *tet*A, *tet*B, and *erm*B) and primer annealing (54˚C for 40 sec. to bla_{SHV}, $bla_{CTX\text{-}M}$, $bla_{\text{OXA-1}}$, & bla_{TEM}), then final extension at 72˚C for 10 min. All amplified PCR products were electrophoresed on 1.5% agarose (Sigma-Aldrich, Co., St. Louis, MO, USA) in 100 ml TBE buffer, then stained with 0.5 µg/ml Ethedium bromide. Under the ultraviolet Tran illuminator, the gel was imaged.

Laboratory for veterinary quality control in poultry production, Animal Health Research Institute.

Results

Phenotypic characters and microscopically examination of Edwardsiella tarda in fish

All of the obtained isolates were gram-negative, motile, and short rods, according to a bacteriological analysis by Gram's stain. On Salmonella-Shigella agar, the isolates developed effectively and produced characteristic small transparent colonies with black centers due to H_2S production that varied in size (1–3 mm in diameter) and raised-shaped colonies as in (Fig. 1). Biochemically, isolates with an oxidase test negative (no color) were carried by microbact identification kits for GNB (gram-negative bacteria), as the overall incidence of *E. tarda* amongst the fish studied was 28% confirmed by microbact.

Molecular confirmation of E. tarda

Molecular confirmation of *E. tarda* by polymerase chain reaction using species-specific primers targeting a 415 bp fragment of the *gyrB*1 gene showed 12 (85.7%) isolates were positive from 14 examined isolates.

(Fig. 2).

E. tarda isolates' Antibiogram

The obtained isolates of *E. tarda* were tested for antimicrobial susceptibility (Fig. 3). (n=12). The isolates that were screened were extremely sensitive to ciprofloxacin (0/12, 0%), while they had complete resistance to erythromycin (12/12, 100%), followed by highly resistant against tetracycline (TE) (10/12, 83.3%), amoxicillin (10/12, 83.3%), streptomycin (7/12, 58.3%), ampicillin (7/12, 58.3%), and moderate resistance against sulbactam/ampicillin (5/12, 41.6%) and cefotaxime (5/12, 41.6%), and also lower resistance against amoxicillin/Clavulanic acid (2/12, 16.6%), while the lowest resistance against norfloxacin (1/12, 8.3%).

AMR genes Distribution amongst E. tarda isolates

In terms of antibiotic resistance genes found in *E. tarda* isolates, PCR confirmed the presence of bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CTX-M}, *tetA, tetB,* and *ermB* resistance genes at 85.7*%,* 14.2*%,* 0*%,* 0*%,* 57.1*%,* 42.8%, and 0*%,* respectively (Table 2 and Figs. 4, 5, 6).

Discussion

Overuse of antibiotics can lead to strains of bacteria that are immune to the drug's effects.

Dangerous antibiotics pose a threat to public health. In fish farms, the unchecked use of antibiotics for the prevention or treatment of Edwardsiellosis has been linked to an increase in antibiotic resistance & the spread of resistance genes to other bacteria [17]. In this study, clinical examination and p.m. in the collected sample resembled those obtained by [29] and [30]. There was a twenty-four percent summertime increase in the rate of *Edwardsiella tarda* isolation. In addition, the size of the colonies on S. S agar media varied from small clear colonies with black centers to mostly black colonies, allowing for a visual distinction between the isolated *E. tarda* strain along with other colonies, which agreed well with the results of the phenotypic and genotypic tests [31]. Biochemically, the isolated strains show variation in citrate utilization, and this result agrees with [32], [33], and disagrees with [34]. *Edwardsiella tarda* can be distinguished from other *Enterobacteriaceae* bacteria based on both phenotypic and biochemical characteristics [35]. However, numerous trials demonstrated that *E. tarda* isolates display substantial phenotypic as well as biochemical diversity [36, 37]. These distinctions were associated with whether or not the plasmid that controls metabolic traits in bacteria was present, or with whether or not the bacteria could grow exclusively on citrate as a source of energy & carbon. [38].

An accurate and rapid test for identification of *Edwardsiella tarda* in diseased fish is PCR by detecting the *gyrB1* gene, which is specific for the

identification & virulence of *E. tarda* isolates [39, 40]. *GyrB* is more trustworthy than *16s rRNA* for detecting relationships between closely related bacterial species [10]. In this study, the use of a *gyrB1* gene primer proved that biochemical identification of isolates was positive for *E. tarda gyrB* gene by 85% (12 isolates were positive for *E. tarda gyrB1* gene from 14 isolates), and this percent (accuracy of microbact) agrees with [41], so *E. tarda was* identified by both biochemical tests (Microbact™ Oxoid Australia) and PCR by identifying the *gyrB1* gene (a taxonomic marker for virulent *E. tarda*) at 415 bp, which agrees with [31, 42].

Antimicrobial susceptibility tests revealed a substantial difference in susceptibility among the *E. tarda* isolates examined. All of the isolates were susceptible to ciprofloxacin and norfloxacin (except isolate No. 10, which is entirely resistant to norfloxacin), and they demonstrated multidrug resistance to tetracycline, macrolides, at least one antibiotic belonging to penicillin, cephalosporin, and aminoglycosides, which agree with [43]. Natural sensitivity to quinolones along with natural resistance to macrolides have been documented for *E. tarda* [44], as well as these findings are in close agreement with those reports. while, over time, had developed a resistance to virtually every class of antibiotics [7]. The unchecked administration of antibiotics in fish farms for the purpose of preventing or treating Edwardsiellosis has led to an increase of antibiotic resistance & the spread of resistance genes to other bacteria; these genes are related with virulence factors of the pathogen [17]. [44] and [45] recorded that most of the *E. tarda* isolates show multidrug resistance. These isolates, when they cause disease, may be difficult to control. Multiple antibiotic-resistant *E. tarda infections* in tilapia were recorded [46]. So, treatment of Edwardsiellosis in multidrug-resistant isolates by antibiotics was difficult.

The antibiotic resistance profile of the isolated *E. tarda* was nearly consistent with other authors, including [47] in resistance to streptomycin as well as tetracycline, [48] in observing amoxicillin-resistant strains that were susceptible to ciprofloxacin, along with [45] in observing a high frequency of resistant isolates for the β - lactams (cefotaxime, ampicillin). In this study, *E. tarda* shows high resistance to tetracycline, and this is due to its widespread application for the promotion of growth in aquaculture and livestock. Tetracycline resistance genes in fish bacteria were shown to be like those found in human isolates, according to [49]. Other studies recorded that *E. tarda* strains are tetracycline resistant, with multidrug-resistant genes [30, 50].

In this study, antibiotic resistance genes were screened and characterized using multiplex and duplex PCR in seven *E. tarda* isolates. The results were identified as follows: bla_{TEM}, bla_{SHV}, $bla_{\text{OXA-1}}$ and $bla_{\text{CTX-M}}$ in 85.71%, 14.28%, 0%, and 0% isolates, respectively. Tetracycline resistance genes were identified as follows: *tetA* (A) in 57.14% and *tetB* in 42.85%. and erythromycin resistance gene (*ermB*) were not detected, which is in close agreement with [50], who recorded that bla_{TEM} (80%), *tetA* (60%), and *ermB* were not detected, and with [45], which recorded that bla_{OXA} not detected, and disagrees with [30], which records bla_{CTX} (86.4%). This study demonstrated that fish are potential sources of multidrug-resistant *E. tarda* for humans. The majority of Egyptian laboratories lack the resources to do quick diagnosis and molecular testing, & this trial has shed light on the resulting information gaps as well as limits in the country's ability to monitor antimicrobial drug resistance. Understanding the trends in antimicrobial drug resistance in our country, increasing the capacity of

laboratories in addition their personnel to accurately and promptly identify antimicrobial resistance to drugs, along with introducing newer antimicrobials to target emerging resistance mechanisms in gram-negative species are all areas that will be prioritized in future efforts to address at the national level.

Conclusions

This study demonstrated that fish are potential sources of multidrug-resistant *E. tarda* and proved that *E. tarda* isolates were virulent with multiple antibiotic resistance (MAR); they are fish pathogens of public health importance. So, the spread of resistance to humans may occur, leading to treatment failure. We recommended a reduction and restriction in the use of antibiotics in fish farms. The emergence of multidrug-resistant *E. tarda* isolates poses a threat to Egypt's fish farming industry. The most important tool for detecting *E. tarda* and controlling septicemic illnesses in aquaculture is molecular-based detection.

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Conflict of interest:

The authors declared no conflict of interest.

Funding statement:

My own money

Author`s contribution:

Amal Fathy Yousef: sampling, isolation, biochemical identification of *E. tarda* and antimicrobial susceptibility Amgad Ahmed Moawad: isolation and identification of *E. tarda*
Mostafa Safwat Abdou: biochemical and PCR identification of *E. tarda* and antimicrobial susceptibility

Ethical approval:

All fish handling was conducted under the guidelines for the care and use of animals for scientific purposes established by the Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

TABLE 1. Oligonucleotide primers sequences used in present study

Gene	Sequence (5′….. 3′)	Size	Reference
E. tarda gyrB1	F; GCATGGAGACCTTCAGCAAT R; GCGGAGATTTTGCTCTTCTT	415 bp	[22]
***tet*A**	F; GGTTCACTCGAACGACGTCA R; CTGTCCGACAAGTTGCATGA	576 bp	[24]
***tet*B**	F; CCTTATCATGCCAGTCTTGC R; ACTGCCGTTTTTTCGCC	773 bp	[25]
***erm*B**	F; CATTTAACGACGAAACTGGC R; GGAACATCTGTGGTATGGCG	425 bp	[26]
$bla_{\text{CTX-M}}$	F; ATGTGCAGYACCAGTAARGTKATG GC R; TGGGTRAARTARGTSACCAGAAYCAGCGG	593 bp	[27]
bla_{SHV}	F; ′AGGATTGACTGCCTTTTTG′ R; ′ATTTGCTGATTTCGCTCG′	392 bp	
bla_{OXA-1}	F; ′ATATCTCTACTGTTGCATCTCC′ R; ′AAACCCTTCAAACCATCC′	619 bp	[28]
bla_{TEM}	F; ′ATCAGCAATAAACCAGC′ R; ′CCCCGAAGAACGTTTTC′	516 bp	

Fig. 1. Colonial characters of *Edwardsiella tarda* on SS agar

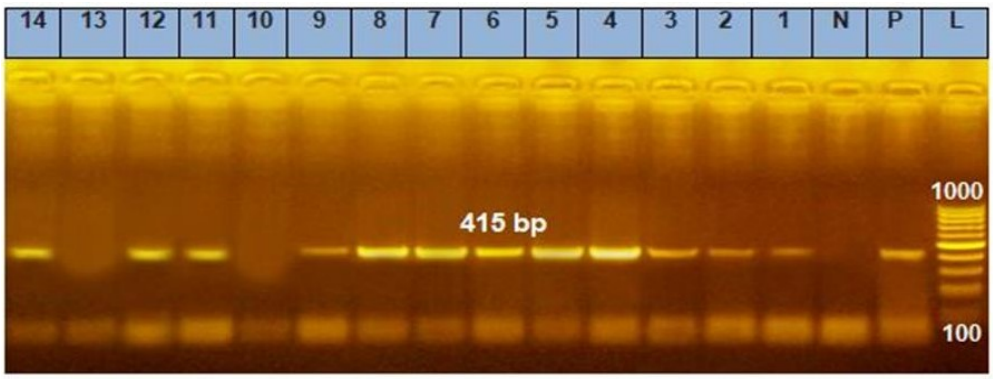

Fig. 2. Agarose gel electrophoresis patterns of the PCR product of the amplicon (415 bp) of *gyrB*1. Lane L: 100-bp DNA ladder as a molecular-size DNA marker; Lane P: positive control of the amplicon (415 bp) for *gyrB*1; Lane N: negative control (deionized water) for *gyrB*1; Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, and 14: analyzed samples of *E. tarda* isolates showing the positive 415 bp amplicon of *gyrB*1.

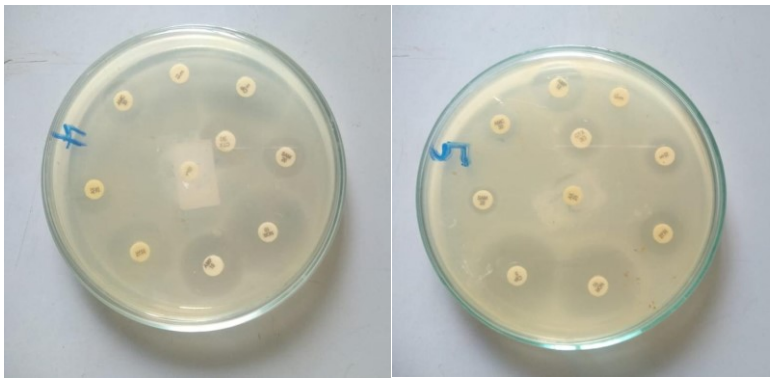

Fig. 3. Antimicrobial susceptibility

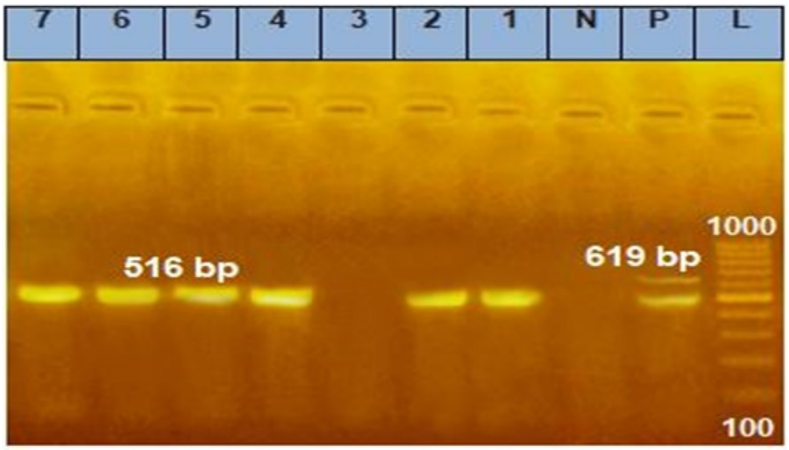

Fig. 4. Agarose gel electrophoresis of duplex PCR of *bla*$_{TEM}$, and *bla*$_{OXA}$ (516 and 619 bp, respectively) resistance genes for characterization of *E. tarda*. Lane L: 100 bp DNA ladder as a molecular-size DNA marker; Lane P: positive control (516 and 619 bp, respectively) for *bla*$_{TEM}$, and *bla*$_{OXA}$; Lane N: negative control (deionized water) for *bla*$_{OXA}$ and *bla*$_{TEM}$; Lanes 1, 2, 4, 5, 6 and 7: positive isolates for *bla*$_{TEM}$ (at 516 bp in samples), but all samples were negative for the *bla*$_{OXA}$ gene at 619 bp.

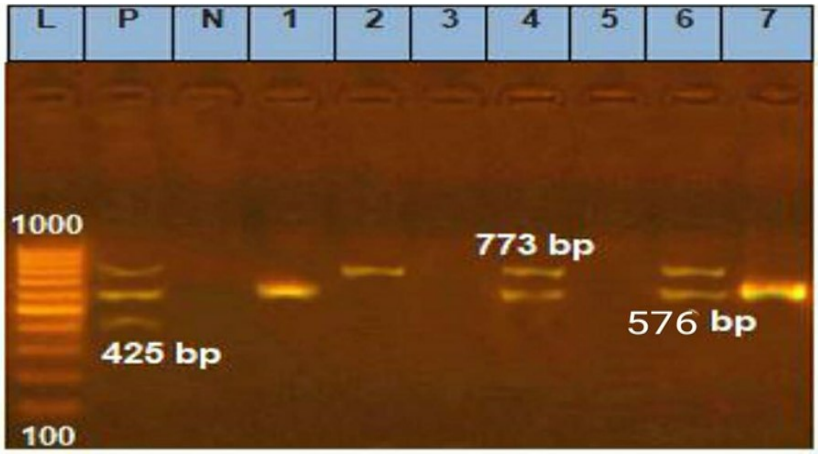

Fig. 5. Agarose gel electrophoresis of multiplex PCR of *tetA*, *tetB*, and *ermB* (576, 773, and 425 bp, respectively) resistance genes for characterization of *E. tarda*. Lane L: 100 bp DNA ladder as a molecular-size DNA marker; Lane P: positive control at (576, 773, and 425 bp, respectively) for *tetA*, *tetB*, and *ermB*; Lane N: negative control (deionized water) for *tetA*, *tetB*, and *ermB*; Lanes 1, 4, 6 and 7: positive isolates for *tetA* (at 576 bp in samples); Lanes 2, 4, and 6: positive isolates for *tetB* (at 773 bp in samples); but all samples were negative for *ermB* gene at 425 bp.

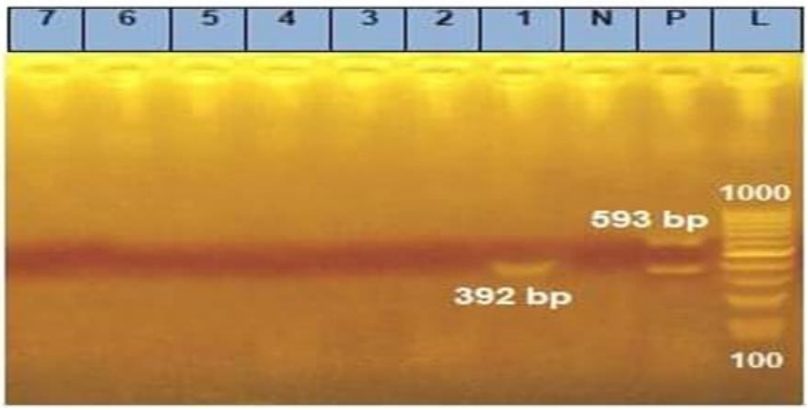

Fig. 6. Agarose gel electrophoresis of duplex polymerase chain reaction of *bla*$_{SHV}$, and *bla*$_{CTX-M}$ (392 and 593 bp, respectively) resistance genes for characterization of *E. tarda*. Lane L: 100 bp DNA ladder as a molecular-size DNA marker; Lane P: positive control at (392 and 593 bp, respectively) for *bla*$_{SHV}$, and *bla*$_{CTX-M}$; Lane N: negative control (deionized water) for *bla*$_{SHV}$, and *bla*$_{CTX-M}$; Lanes 1: positive isolates for *bla*$_{SHV}$ (at 392 bp in samples), but all samples were negative for the *bla*$_{CTX-M}$ gene at 593 bp.

TABLE 2. Result of duplex and multiplex PCR for 7 antibiotic resistant genes.

Sample no.	*bla*$_{TEM}$	*bla*$_{OXA}$	*bla*$_{SHV}$	*bla*$_{CTX-M}$	*tetA*	*tet(B)*	*ermB*
1	+	-	+	-	+	-	-
2	+	-	-	-	-	+	-
3	-	-	-	-	-	-	-
4	+	-	-	-	+	+	-
5	+	-	-	-	-	-	-
6	+	-	-	-	+	+	-
7	+	-	-	-	+	-	-

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التوصيف الجزيئي لمقاومة مضادات الميكروبات في بكتريا ادواردسيلا تاردا المعزولة من أسماك البلطي المريضة في مصر

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ادواردسيلا تاردا هي بكتريا حيوانية المنشأ تسبب داء الحويصلات ، وتتميز بنفوق واسع في الأسماك مما يؤدي إلى خسائر اقتصادية عالية، ولا تتوفر سوى معلومات قليلة عن التوصيف الجزيئي لمقاومة مضادات الميكروبات في *E. tarda*. لذلك ، كان الهدف من هذا البحث هو تصوير مقاومة البكتيريا *E. tarda* المعزولة من أسماك البلطي في مصر على المستوى الجزيئي والتي تم تحديدها من خلال كل من الاختبارات الكيميائية الحيوية (Microbact ™) و PCR باستخدام بادئات خاصة بالأنواع تستهدف *gyrB*1 (*gyrase* B1) الجين (علامة تصنيفية لـ *E. tarda*) عند 415 نقطة أساس. تم عزل 24 عزلة من *E. tarda* من 100 عينة سمكية مريضة جمعت عشوائياً من 10 مزارع أسماك خاصة (10 أسماك لكل منها) في محافظة كفر الشيخ ، مصر. تم تحديد حساسية جميع العزلات ضد 10 عوامل مضادة للميكروبات (أموكسيسيلين (AMX) ، أمبيسلين (AMP) ، سيبروفلوكساسين (CIP) ، تتراسيكلين (TE) ، سيفوتاكسيم (CTX) ، ستربتومايسين (S) ، إريثروميسين (E) ، نورفلوكساسين. / Clavulanic acid و sulbactam / ampicillin) حيث أظهرت معظم هذه العزلات أنماطًا ظاهرية مقاومة للأدوية المتعددة حيث كانت المقاومة الكاملة ضد الإريثروميسين (100٪) ثم أعلى مقاومة ضد التتراسيكلين والأموكسيسيلين (83.3٪). تم فحص الجينات المقاومة للمضادات الحيوية وتوصيفها باستخدام PCR. تم اكتشاف جينات ترميز β-Lactamase على النحو التالي: bla_{TEM} و bla_{SHV} في عزلات 85.7٪ و 14.2٪ على التوالي. بينما لم يتم اكتشاف bla_{OXA} و bla_{CTX-M} ، تم تحديد جينات مقاومة التتراسيكلين على النحو التالي: *tet*A في 57.1٪ و *tet*B في 42.8٪ ، ولكن لم يتم اكتشاف جين مقاومة الاريثروميسين (*erm*B). أظهرت هذه الدراسة أن الأسماك هي مصادر محتملة لبكتيريا *E. tarda* المقاومة للأدوية المتعددة.

الكلمات الدالة: دواردسيلا تاردا (E. تاردا) ؛ gyrB1؛ جينات مقاومة - مضادات الميكروبات- أسماك- ميكروباكت™