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# Molecular characterization and antimicrobial effect of some antibiotic on *Yersinia enterocolitica* isolated from different sources at Kaliobia, Egypt.

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**ABSTRACT**

Antimicrobial resistance to antibiotics is a major barrier in treating serious nosocomial infections. There are some studies evaluating the resistance profile of the *Y. enterocolitica* strains from various countries. Therefore, this study aims evaluating the 31 *Y. enterocolitica* isolates in terms of their antimicrobial resistance. They were previously isolated by the same authors from 225 random samples of cow milk, beef, chicken meat, diarrheic cow feces, and diarrheic human stool of patients suffering from vomiting and diarrhea that were collected from different shops, dairy herds, and hospitals (45 for each), at Kaliobia Governorate of Egypt, besides detection of some antibiotic's resistant genes in some strains. This study cleared that the isolates were extremely resisting oxacillin, then tetracycline, Nalidixic acid, and cefoxitin. Meanwhile, they were extremely sensitive to norfloxacin, then ciprofloxacin, cefotaxime, and gentamycin. Moreover, PCR appeared that $aad_{A1}$, $tet_{A(A)}$, $qnr_{A,}$ and $bla_{CTX-M}$ genes were detected in all eight studied *Y. enterocolitica* isolates. So, the study concluded that the antimicrobial-resistant *Y. enterocolitica* strains in animal-origin foods could be a public health concern for consumers. They could also be considered for antimicrobial resistance control and food safety measures.

## 1. INTRODUCTION

*Y. enterocolitica* is a psychotropic food and waterborne, Gram-negative, facultative anaerobic bacteria that have global interest. It is a family *Enterobacteriaceae* member and can cause severe gastrointestinal infections (Nesbakken et al., 2015 and Bancerz-Kisiel *et al*., 2018). It is extremely heterogeneous, with over 50 serotypes and six biotypes (1A, 1B, 2, 3, 4, and 5). The biotypes pathogenic properties, ecological niches, and geographical distribution vary (Sharma *et al*., 2006 and Peruzy *et al*., 2017). It has been found that *Yersinia enterocolitica* is very tolerant to most antibiotics, with the exception of ampicillin, penicillin, first-generation cephalosporins, and amoxicillin-clavulanic acid (Soltan-Dallal *et al*., 2010; Bolton *et al*. 2013 and Bonardi *et al*. 2018). The resistance levels depend on the strain type and temperature (Bottone *et al*., 2005). However, the antibiotic over-usage in animal and poultry farms and the spread of antimicrobial-resistance bacterial genes across various species have led to identifying the *Y. enterocolitica* drug-resistant strains in food and the environment (Musavian et *al*., 2014; Özdemir and Arslan, 2015 and Ye *et al*., 2016). Additionally, the antimicrobial resistance results in treatment failures necessitating the usage of expensive and/ or toxic alternative medications that are more expensive in the majority of cases. The antibiotic resistance spread among *Y. enterocolitica* is also a public health problem (Pandove *et al*., 2012). Thus, animal-origin foods, especially poultry meat, beef, milk, and their products, are regarded a crucial vector for the *Y. enterocolitica* transmission with antimicrobial resistance to humans as a result of the improper handling and cooking during preparation resulting in considerable health problems for consumers, particularly the young and newborns (Bonardi *et al*., 2016). In addition, as previously highlighted, determining the antimicrobial resistance and detecting virulence genes as chromosomal changes result or the genetic material interchange via plasmids and transposons are very important (Li and Fanning, 2017). In terms of prevalence, the antimicrobial-resistant and multidrug-resistant *Y. enterocolitica* strains have increased steadily (Bonardi *et al*., 2018). The antimicrobial-resistant *Y. enterocolitica* spread in animal and poultry products remains poorly characterized in Egypt. So, this research aimed evaluating the antimicrobial resistance of 31 *Y.enterocolitica* isolates (previously isolated by the same authors from 225 random samples of cow milk, beef, chicken meat, diarrheic cow feces, and diarrheic human stools of patients suffering from vomiting and diarrhea) collected from different shops, dairy herds, and hospitals at Kaliobia Governorate Egypt, besides detecting some antibiotic-resistant virulence genes in some strains.

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## 2. MATERIAL AND METHODS

*2.1. Samples:*

Thirty-one *Y. enterocolitica* strains were studied. These strains were previously isolated and identified in the present study from 225 random samples of cow milk, beef, chicken meat, diarrheic cow feces, and diarrheic human stool of patients suffering from vomiting and diarrhea. They were collected from different shops, dairy herds, and hospitals (45 for each) at Kaliobia Governorate, Egypt, following Markey *et al*. 2013 and ISO 10273 2003.

*2.2. Invitro anti-microbial sensitivity test:*

In terms of antimicrobial sensitivity, the 31 studied *Y. enterocolitica* isolates were tested against different 13 antimicrobials from distinct classes by Kirby-Bauer disk diffusion technique using Muller-Hinton agar (Oxoid) plates in accordance with the CLSI 2018 guidelines. The utilized antimicrobial standardized disks (Oxoid) were {cefotaxime (CTX/30), cefoxitin (FOX/30), gentamicin (GEN/10), co-trimoxazole (COT/25), ciprofloxacin (CIP/5), doxycycline (DO/30), norfloxacin (NOR/10), Nalidixic acid (NA/30), oxacillin (OX1), streptomycin (S/10), and tetracycline (TE/30).

*2.3. Molecular detection of antibiotic-resistant genes of Y. enterocolitica:*

Genotypic detection of four antibiotic-resistant genes, streptomycin (*aadA1*), tetracycline $tet_{A(A)}$, quinolones ($qnr_A$), and β-lactamase ($bla_{CTX-M}$) which were detected in eight random *Y. enterocolitica* (two from cow milk, chicken meat, beef and one from cow feces and human stool) that showed antibiotic-resistant by disk diffusion method to the same studied isolates using polymerase chain reaction, after QIAamp® DNA mini kits guidelines (Germany, GmbH, Qiagen), Emerald Amp GT PCR mastermix (Japan, Takara), and 1.5% agarose gel electrophoreses (Sambrook *et al.*,1989) by the usage of the target genes, primers sequences, cycling conditions, and amplicons sizes as Table (1) shows.

Table 1 Primer sequences of target genes, cycling conditions, and amplicons

| Target gene | | Primer sequences (5'-3') | Amplified segment (bp.) | Primary denaturation | Amplification (35 cycles) | | | Final extension | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Secondary denaturation | Annealing | Extension | | |
| $aad_{A1}$ | F | TATCAGAGGTAGTTGGCGTCAT | 484 bp | 94˚C 5min. | 94˚C 30sec. | 54˚C 40sec. | 72˚C 45sec. | 72˚C 10min. | Randall *et al.,2004* |
| | R | GTTCCATAGCGTTAAGGTTTCATT | | | | | | | |
| $tet_{A(A)}$ | F | GGTTCACTCGAACGACGTCA | 576 bp. | 94˚C | 94˚C | 50˚C 40sec. | 72˚C | 72˚C | |
| | R | CTGTCCGACAAGTTGCATGA | | 5min. | 30sec. | | 45sec. | 10min. | |
| $qnr_A$ | F | ATTTCTCACGCCAGGATTTG | 516 bp. | 94˚C 5min. | 94˚C | 55˚C 40sec | 72˚C 45sec. | 72˚C | Robicsek *et* |
| | R | GATCGGCAAAGGTTAGGTCA | | | 30sec. | | | 10min. | *al.*, 2006 |
| *BlaCTX-M* | F | ATG TGC AGY ACC AGT AAR GTK ATG GC | 593 bp. | 94˚C 5 min. | 94˚C 30 sec. | 54˚C 40sec. | 72˚C 45sec. | 72˚C 10min. | Archambault *et al.*, 2006 |
| | R | TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | | | | | | | |

## 3. RESULTS

For the studied *Y. enterocolitica* invitro (Table. 2), the sensitivity test findings demonstrated that the isolates were extremely resisting oxacillin (90.3%), tetracycline (80.7%), nalidixic acid (61.3%), and cefoxitin (58.1%). Meanwhile, they were intermediate sensitive to doxycycline (64.5%); Co- trimoxazole (54.8%), and streptomycin (51.6%). Moreover, they were extremely responsive to norfloxacin (83.9%), ciprofloxacin (77.4%), cefotaxime (67.7%), and gentamycin (64.5%).

Table 2 In-Vitro anti-microbial Sensitivity test for 31 studied Y. enterocolitica.

| Antimicrobial agents | Disk concentrations | Sensitive | | Intermediate | | Resistant | | AA |
|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | |
| Oxacillin | 1 µg | 1 | 3.2 | 2 | 6.5 | 28 | 90.3 | R |
| Tetracycline | 30 µg | 1 | 3.2 | 5 | 16.1 | 25 | 80.7 | R |
| Nalidixic acid | 30 µg | 1 | 3.2 | 11 | 35.5 | 19 | 61.3 | R |
| Cefoxitin | 30 µg | 5 | 16.1 | 8 | 25.8 | 18 | 58.1 | R |
| Doxycycline | 30 µg | 5 | 16.1 | 20 | 64.5 | 6 | 19.4 | IS |
| Co-trimoxazole | (1.25/23.75) mcg | 3 | 9.7 | 17 | 54.8 | 11 | 35.5 | IS |
| Streptomycin | S/10 | 2 | 6.5 | 16 | 51.6 | 13 | 41.9 | IS |
| Norfloxacin | 10 µg | 26 | 83.9 | 4 | 12.9 | 1 | 3.2 | S |
| Ciprofloxacin | 5 µg | 24 | 77.4 | 5 | 16.1 | 2 | 6.5 | S |
| Cefotaxime | 30 µg | 21 | 67.7 | 7 | 22.6 | 3 | 9.7 | S |
| Gentamicin | 10 µg | 20 | 64.5 | 8 | 25.8 | 3 | 9.7 | S |

No.: Isolates number. AA: Antibiogram activity.
%: The ratio of the studied *Y. enterocolitica* total number (31).

The results of genotyping detection of antibiotic-resistant genes showed that $aad_{A1}$, $tet_{A(A)}$, $qnr_A$, and $bla_{CTX-M}$ antibiotic-resistant genes were amplified in all eight studied strains giving products of 484 bp. for $aad_{A1}$, 576 bp. for $tet_{A(A)}$, 576 bp. for $qnr_A$, and 593 bp. for $bla_{CTX-M}$ (Figures, 1-4).

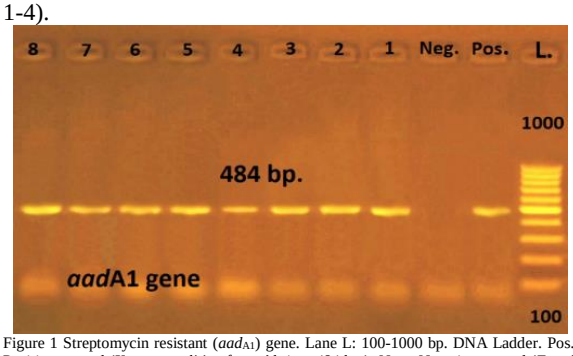


Figure 1 Streptomycin resistant ($aad_{A1}$) gene. Lane L: 100-1000 bp. DNA Ladder. Pos.: Positive control (*Y. enterocolitica* form Ahri. at 484 bp.). Neg.: Negative control (*E. coli* AJ413986). Lanes 1-8: Positive *Y. enterocolitica* (1&2 cow milk;3&4 chicken meat;5&6 beef;7 cow feces and 8 human stool).

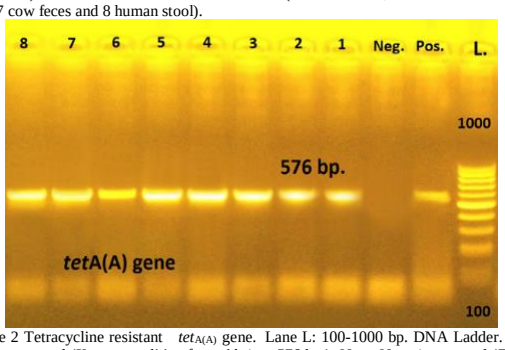


Figure 2 Tetracycline resistant $tet_{A(A)}$ gene. Lane L: 100-1000 bp. DNA Ladder. Pos.: Positive control (*Y. enterocolitica* form Ahri. at 576 bp.). Neg.: Negative control (*E. coli* AJ413986). Lanes 1-8: Positive *Y. enterocolitica* (1&2 cow milk;3&4 chicken meat;5&6 beef;7 cow feces and 8 human stool).

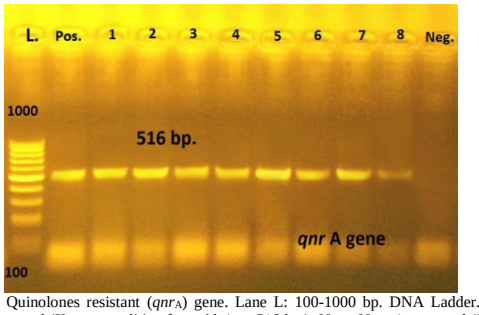


Figure 3 Quinolones resistant ($qnr_A$) gene. Lane L: 100-1000 bp. DNA Ladder. Pos.: Positive control (*Y. enterocolitica* form Ahri. at 516 bp.). Neg.: Negative control (*E. coli* AJ413986). Lanes 1-8: Positive *Y. enterocolitica* (1&2 cow milk; 3&4 chicken meat; 5&6 beef; 7 cow feces and 8 human stool).

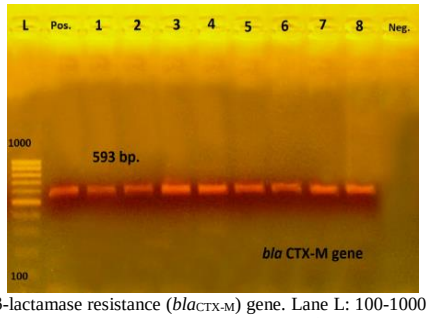


Figure 4 β-lactamase resistance ($bla_{CTX-M}$) gene. Lane L: 100-1000 bp. DNA Ladder. Pos.: Positive control (*Y. enterocolitica* form Ahri. at 593 bp.). Neg.: Negative control (*E. coli* AJ413986). Lanes 1-8: Positive *Y. enterocolitica* (1&2 cow milk; 3&4 chicken meat; 5&6 beef; 7 cow feces and 8 human stools).

## 4. DISCUSSION

*Y. enterocolitica* is a gram-negative bacterium that cause food poisoning. It spreads widely in water, the environment, dairy products, and meats. Their presence, especially the antimicrobial-resistant ones with high levels, indicates a potential risk of producing yersiniosis infection in humans and animals (Jamali et al., 2015 and Penga et al., 2018). Among the pathogenic strains, the antibiotic resistance has increased worldwide, especially *Y. enterocolitica* (Tavassoli et al., 2018). This study findings for the 31 *Y. enterocolitica* isolates in terms of antimicrobial sensitivity (Table 2) revealed that they were extremely resisting oxacillin, then tetracycline, Nalidixic acid, and cefoxitin. Quite comparable results were recognized by Aghamohammad et al. 2015; Bharathy et al. 2015; Ozdemir and Arslan 2015: Ye et al. 2016; Penga et al. (2018); Verbikova et al. (2018); Abd El Tawab et al. (2021) and Younis et al. (2021). Moreover, they were intermediate sensitive to doxycycline, Co- trimoxazole, and streptomycin, but they were extremely sensitive to norfloxacin, then ciprofloxacin, cefotaxime, and gentamycin, with lower resistance to these antimicrobial agents. These results came in harmony with those obtained by Hadef et al. (2015), Penga et al. (2018); Verbikova et al. (2018); Younis et al. (2019), and Abd El Tawab et al. (2021).

The findings demonstrated that phenotypic and multiple antibiotic resistances (MDR) are broadly spread across the 31 studied *Y. enterocolitica* isolates. The *Y. enterocolitica* drug resistance was unrelated to their source origin or biotypes. Similar results were recognized by other researchers (Bonardi et al., (2010); Penga et al., (2018) and Younis et al., (2019)) who concluded that the inappropriate antibiotics usage in Egypt and other countries could be the primary reason for high resistance rates in these isolates. Thus, some actions must be done to minimize this problem, such as regulating the antibiotic usage, increasing the research scope to comprehend the drug resistance genetic mechanisms, and doing extra efforts to develop new drugs. The *Y. enterocolitica* antimicrobial resistance is continuously evolving, and the horizontal gene transfer via plasmids is the key function (Sharma et al., (2004); Rozwandowicz et al., (2018) and Penga et al., (2018)). In Egypt, a few research on *Y. enterocolitica* resistance have been mentioned in literature, focusing on the genes associated with producing β-lactamases, streptomycin, quinolones, and tetracycline-resistant genes. So, the present study was directed for recognizing four antibiotic-resistant gene genes (aadA1, tetA (A), qnrA, and blaCTX-M) on eight randomly isolated *Y. enterocolitica* from different sources showed antibiotic-resistant by disk diffusion method to the same studied isolates by using PCR. The results of PCR cleared that all these antibiotic-resistant virulence genes were detected in all eight studied *Y. enterocolitica* isolates, where the streptomycin-resistant gene ($aad_{A1}$) was amplified at 484 bp. (Fig. 1), tetracycline-resistant gene $tet_{A(A)}$ was amplified at 576 bp. (Fig. 2), quinolones resistant gene ($qnr_A$) gene was amplified at 516 bp. (Fig. 3), and β-lactamase resistance gene (blaCTX-M) was amplified at 593 bp. (Fig. 4). The same genes were detected in *Y. enterocolitica* strains isolated from milk, beef, chicken meat, cow feces, and foodborne outbreaks as stated by Randall et al.( 2004) and Karlsson et al. (2021) for the $aad_{A1}$ gene; Randall et al.( 2004), Penga et al.( 2018), Gkouletsos et al.(2019), Younis et al. (2019) and Karlsson et al. (2021) for the $tet_{A(A)}$ gene; Robicsek et al.( 2006), Bonke et al.( 2011), Penga et al.( 2018), Younis et al. (2019) for the $qnr_A$ gene and Archambault et al.( 2006), Bonke et al.( 2011), Ye et al. (2015), Zamzam, Nour (2017), Penga et al.( 2018) for the $bla_{CTX-M}$ gene. So, the presence of $aad_{A1}$, $tet_{A(A)}$, $qnr_A$, and $bla_{CTX-M}$) genes and the phenotypic resistance to the antibiotics of these groups were positively correlated.

Finally, the results of the author stated that multiple antibiotic resistances are broadly spread across the 31 studied *Y. enterocolitica* isolates and decided the fact of McDermott et al. (2002) and Jamali et al. (2015) that the antibiotics application in animal food to control and treat infectious diseases in dairy and poultry farms can be regarded the primary method through which the antibiotic-resistant bacteria are transmitted from animals to humans.

## 5. CONCLUSION

In conclusion, the antimicrobial-resistant *Y. enterocolitica* strains in milk, beef, chicken meat, and cow feces could be a public health concern for consumers. Therefore, continuous monitoring of the *Y. enterocolitica* isolates antimicrobial resistance in human and animal foods is required to prevent public health risks.

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