Original Paper

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

Ashraf, A. Abd El Tawab\(^1\), Ahmed, A. A. Maarouf\(^2\), Reham, S.M. Darwish\(^1\) and Enas A. Soliman\(^1\)

\(^1\)Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, Kaliobia, Egypt.  
\(^2\)Animal Research Institute, Benha Branch.

**ARTICLE INFO**  
**Keywords**  
*Yersinia enterocolitica*  
diarrheic human stool  
Molecular characterization  
antibiotic-resistant genes

**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 cefotixin. Meanwhile, they were extremely sensitive to norfloxacin, then ciprofloxacin, cefotaxine, and gentamyacin. Moreover, PCR appeared that *aadA4*, *tet(A)*, *qnrA*, and *blacTEM* 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-Kiszeli et al., 2018). It is extremely heterogeneous, with over 20 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.

* Corresponding author: rehamsaiddarwish@gmail.com
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. In vitro anti-microbial sensitivity test:
In terms of antimicrobial sensitivity, the 31 studied Y. enterocolitica isolates were tested against different 13 antimicrobials from different 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 (OX/1), 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 tetA (qnrA), and β-lactamase (blaCTX-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 QH Amp® 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.

3. RESULTS

For the studied Y. enterocolitica invitro (Table 2), the sensitivity test findings demonstrated that the isolates were extremely resistant 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%).

The results of genotyping detection of antibiotic-resistant genes showed that aadA1, tetA (qnrA), and blaCTX-M antibiotic-resistant genes were amplified in all eight studied strains giving products of 484 bp. for aadA1, 576 bp. for tetA, 576 bp. for qnrA, and 593 bp. for blaCTX-M (Figures, 1-4).
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 (aadA1) was amplified at 484 bp. (Fig. 1), tetracycline-resistant gene tet(AA) was amplified at 576 bp. (Fig. 2), quinolones resistant gene (qnrA) 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 aadA1 gene; Randall et al. (2004), Penga et al. (2018), Gkouletsos et al. (2019), Younis et al. (2019) and Karlsson et al. (2021) for the tetAA gene; Robicsek et al. (2006), Bonke et al. (2011), Penga et al. (2018), Younis et al. (2019) for the qnrA gene and Archambault et al. (2006), Bonke et al. (2011), Ye et al. (2015), Zamzam, Nour (2017), Penga et al. (2018) for the blaCTX-M gene. So, the presence of aadA1, tetAA, qnrA, and blaCTX-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.

6. REFERENCES


ot resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salm
fying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat. Med., 12:83-
88.
30. Rozwandowicz, M., Brouwer, M.S.M., Fischer, J., Wagenaar, J.A., Gonzalez-Zorn, B., Guerra, B.,