Bulgarian Journal of Veterinary Medicine, 2019 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2019-0044

Original article

PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY, AND VIRULENCE FACTORS OF *YERSINIA ENTEROCOLITICA* ISOLATED FROM RAW MILK IN BASRAH, IRAQ

D. M. KHALID & B. A. ABBAS

College of Veterinary Medicine, University of Basrah, Iraq

Summary

Khalid, D. M. & B. A. Abbas, 2019. Prevalence, antibiotic susceptibility, and virulence factors of *Yersinia enterocolitica* isolated from raw milk in Basrah, Iraq. *Bulg. J. Vet. Med.* (online first).

A total of one hundred and fifty cow, buffalo, and sheep milk samples were collected from several markets in Basrah, Iraq (50 samples from each species). All milk samples were subjected to enrichment in TSB and cold enrichment in PBS, then cultured on YSA agar in order to obtain *Yersinia* species. The highest growth obtained by TSB enrichment was seen in cow milk (24%) followed by buffalo milk (22%) and sheep milk (12%). The results of PBS enrichment showed the highest growth in cow milk (14%) followed by buffalo (8%) and sheep milk (2%). The results showed that both cow and buffalo milk samples were contaminated by *Y. enterocolitica* at 8% while the prevalence in sheep milk was 4%. Ten isolates from different sources in the current study were examined for their susceptibility to 10 antibiotics. The highest susceptibility (100%) was found towards streptomycin, azithromycin and gentamicin, followed by ciprofloxacin and chloramphenicol, 93.3% for each. Low susceptibility was found toward vancomycin (6.66%) and cloxacillin (33.3%). 16S rRNA sequencing showed homology with previously annotated strains at GenBank of National Centre for Biotechnology (NCBI). Multiple sequence alignments exhibited one difference between the sequences at the locus 764. The phylogenic tree of the results demonstrated that the local isolates were closely related to strains previously reported from China. All *Yersinia enterocolitica* strains had the *inv* gene. In contrast, the *ail* gene was found in one strain (10%) while the *yad* gene appeared in 50% of the investigated strains.

Key words: Basrah, buffaloes, cow, milk, sheep, virulence genes, *Yersinia*

INTRODUCTION

Yersinia enterocolitica is a Gram-negative, motile, oxidase-negative and facultatively anaerobic coccobacillus. The colonies are dark and red centered ("bulls eye like") with transparent border on CIN agar. All bacteria of the genus *Yersinia* are catalase-positive, non-spore-forming rods or coccobacilli of 0.5–0.8 × 1–3 μm in size. Strains of *Y. enterocolitica* are urease positive. Most of the strains are motile at 25 °C but non-motile at 37 °C (Cornelis, 1998). These microorganisms

are psychotropic milk-borne enteric pathogens; widely spread in the environment and indigenous to the gastrointestinal tract of warm-blooded animals including dairy cattle (Marshall, 1992).

Chromosome-encoded factors are also needed for pathogenicity. Adherence and invasion to epithelial layers require at least two chromosomal genes, *inv* (invasion) and *ail* (attachment invasion locus) (Miller & Falkow, 1988). The invasion (*inv*) codes for Inv, an outer membrane protein found on the surface of *Yersinia*, which appears to play a vital role in promoting entry into epithelial cells of the ileum during the initial stage of infection, that is responsible for binding to β1-integrins on the apical surface of M cells and initiating uptake of the organism (Schulte *et al.,* 2000). The attachment invasion locus (*ail*) codes for the surface protein Ail, which is produced at 37 °C. In contrast to the *inv*, the *ail* was shown to be restricted only to serotypes associated with the disease (Jourdan *et al.,* 2000). The presence of pYV plasmid (*Yersinia* virulence) with approximately 70,000 bp is also one of the basic indicators of virulence. This plasmid encodes YadA adhesin (i.e., the protein of the external membrane that allows adhering to the host cells), Yop outer proteins, which paralyses the immune system, and Ysc proteins composing the secretion system (Bergann *et al*., 1995; Selma *et al.*, 2006).

Foods of animal origin pose a higher risk of gastrointestinal disease caused by *Y. enterocolitica* in humans. Milk and dairy products are the main consumed foods of animal origin. In addition, several studies have reported the presence of *Y. enterocolitica* in milk and dairy products (Rahimi *et al*., 2013). Antibiotics are commonly used to treat cattle disease especially mastitis in dairy cows and their indiscriminate use leads to the development of multidrug-resistant bacteria rendering antibiotic treatment ineffective (Sadek *et al*., 2014). Antibiotic therapy for the treatment of yersiniosis in humans is not indicated except in systemic and extraintestinal infection and enterocolitis in immunocompromised patients (Mayrhofer *et al*., 2004). The emergence of antimicrobial resistance leads to treatment failures and obligate utilisation of expensive and/or toxic alternative drugs (WHO, 2007). The spread of drug resistance among *Y. enterocolitica* is also of concern for public health. To date, investigation of microbial pathogens in milk in the studied area are available for *Brucella* (Abbas & Aldeewan, 2009; Abbas & Talei, 2010); *E. coli* (Abbas *et al.*, 2012a; Abbas, 2013; Abbas *et al.,* 2013a); *Staphylococcus aureus* (Abbas *et al.*, 2013b; 2014a; 2016), *Listeria monocytogenes* (Abbas & Jaber, 2012) *Bacillus cereus* (Abbas *et al.*, 2012b, 2014b). None of these investigations concern *Yersinia* sp. (Abbas *et al.*, 2017). Therefore, the current study aimed to evaluate the prevalence and resistance of *Y. enterocolitica* in milk samples from three ruminant species.

MATERIALS AND METHODS

Sample collection

In order to obtain *Yersinia* isolates, 150 milk samples were collected between October 2017 and February 2018. A total of 50 samples from cows, buffaloes and sheep were collected from several markets in different parts of Basrah province. All samples were transported in an ice box and kept in the refrigerator in plastic bags until use.

Bacterial isolation

One mL of milk sample was transferred aseptically to 9 mL of Tryptone Soya Broth tubes and incubated at 25 °C for 2 days. For the cold enrichment method, 1 mL of milk was added to 9 mL tubes. Also, 1 mL of milk was directly inoculated into 9 mL PBS tubes. Tubes were incubated for 21 days at 4 ºC (Green Wood & Hooper, 1989; Baharathy *et al.,* 2015). A loopful of cultures were streaked on *Yersinia* selective agar (YSA) plates and incubated for 48 h at 25°C.

The dark red colonies surrounded by a transparent border that resembled bull eye were considered as presumptive *Y. enterocolitica.* Gram stained slides were prepared for these colonies. *Yersinia* appeared as Gram negative non-spore-forming rods or coccobacilli. Biochemical tests including catalase test, oxidase test, Kligler's iron agar test, motility test and indol test were done.

Extraction of bacterial DNA and PCR amplification

For PCR studies, genomic DNA was extracted from bacterial cultures using a commercial kit, and following the protocol provided by the manufacturer (Gene-Aid). Extracted DNA was stored at –20°C until used. Primers used for investigation of 16Sr RNA, *ail, yadA* and *inv* genes of *Y. enterocolitica* are listed in Table 1.

The detection of the PCR amplified products was done by electrophoresis on 1% agarose gel. DNA ladder marker was used to measure the molecular weight of the PCR products. After 30 min, the PCR amplification products were examined under UV light.

DNA sequencing

DNA sequencing of 16s rRNA was done by sending the PCR products for Macrogen Company, South Korea. Basic local Alignment search tool (blast) sequence analysis was performed by blast algorithm for the sequenced results at National Center for Biotechnology. The web site also used for generating a phylogenic tree and multi-sequence alignments.

Antibiotic susceptibility test of Y. enterocolitica isolates

The testing for susceptibility to antibiotics was conducted according to the method of Bauer *et al.* (1966). The antibiotics discs were dispensed onto inoculated Meuller-Hinton plates. The plates were inverted and placed in an incubator at 37 °C for 24 h. Inhibition zones were measured in millimeters using a transparent ruler. The

Table 1. PCR primers used in this study.

Primers	Primer sequence	Product size	References
ail	F: TGGTTATGCGCAAAGCCATGT R: TGGAAGTGGGTTGAATTGCA	356	Feng *et al*., 1992
yadA	F: TAAGATCAGTGTCTCTGCGGCA R: TAGTTATTTGCGATCCCTAGCAC	747	Kapperud *et al.,* 1993
inv	F: CGGTACGGCTCAAGTTAATCTG R: CCGTTCTCCAATGTACGTATCC	183	Thoerner *et al.,* 2003
16s rRNA	F: AGAGTTGATCCTGGCTCAG R: GGTTACCTTGTTACGACTT	1500	Eden *et al*.,.1991

Table 2. Number and percentage of milk samples that showed bacterial growth on YSA medium after TSB and PBS enrichments

Sample types (number)	TSB enrichments	PBS enrichments	Confirmed *Y. enterocolitica*
Cow milk (50)	12 (24%)	7 (14%)	4 (8%)
Buffalo milk (50)	11 (22%)	4 (8%)	4 (8%)
Sheep milk (50)	6 (12%)	1(2%)	2 (4%)
Total (150)	29 (19.3%)	12 (8%)	10 (6.66%)

diameter of the inhibition zone was calculated from the underside of the dish. The results are compared with the minimum inhibition diameter of Bioanalyse Co. (Turkey).

RESULTS

Bacterial isolation

All milk samples were subjected to enrichment in TSB and cold enrichment in PBS, then cultured on YSA agar in order to obtain *Yersinia* species. Multiple types of colonies appeared on YSA which showed less selectivity. The presumptive *Yersinia enterocolitica* colonies isolated from selective medium were subjected to further investigation. Dark and red centered colonies with transparent "bulls eye like" border on YSA were picked and streaked individually on the same agar. By Gram staining, the smears showed Gram negative cocobacilli or different irregular shapes. The results of *Yersinia enterocolitica* biochemical tests showed that this bacterium was catalase positive, oxidase negative, non motile at 37 ^{o}C but motile at 25 ^{o}C.

The results of bacterial isolation and identification are presented in Table 2. According to TSB enrichment results, the highest prevalence with this method was seen in cow milk (24%) followed by buffalo milk (22%) and sheep milk (12%). The results of PBS enrichment showed the higheat prevalence in cow milk (14%) followed by buffalo milk (8%) and sheep milk (2%). All colonies on selective agar with bull eye appearance were subjected to biochemical identification. The results of milk samples showed that both cow and buffalo milk were infected with *Y. enterocolitica* at 8% followed by sheep milk at 4%.

Antibiotic susceptibility of Y. enterocolitica isolates

In this study, ten isolates from different sources were examined for their susceptibility to 10 antibiotics. The highest susceptibility was found toward streptomycin, azithromycin and gentamicin (100% for each), followed by both ciprofloxacin and chloramphenicol (93.3%). A low susceptibility was demonstrated toward vancomycin (6.66%) and cloxacillin (33.3%) (Table 3).

Identification of strains using 16S rRNA

The DNA was extracted from 10 bacterial strains isolated from milk. DNA concentration and purity were determined by nanodrop 1000 spectrophotometer at 260/280 nm.

Table 3. Antibiotics susceptibility of *Yersinia enterocolitica* isolates to different antibiotic discs

Antibiotic	Disc content	Sensitive %	Intermediate %	Resistant %
Vancomycin	30 µg	6.66	0	93.3
Oxytetracycline	10 µg	80	6.66	13.3
Streptomycin	25 µg	100	0	0
Tetracycline	30 µg	80	6.66	13.3
Azithromycin	15 µg	100	0	0
Cloxacillin	25 µg	33.3	20	46.6
Gentamicin	10 µg	100	0	0
Ciprofloxacin	5 µg	93.3	6.66	0
Cefixime	5 µg	60	26.6	13.3
Chloramphenicol	30 µg	93.3	0	6.6

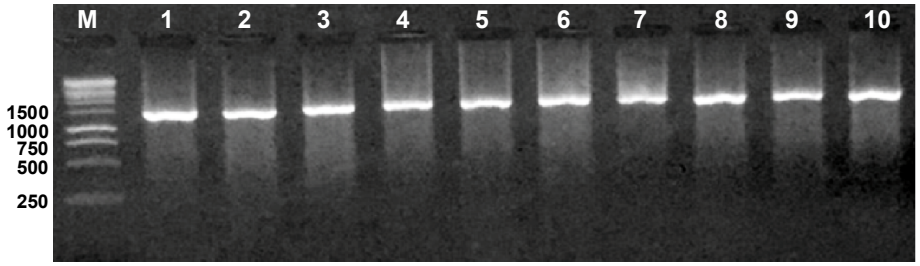

Fig. 1. PCR amplification of 16S rRNA of *Yersinia enterocolitica* isolates showing 1,500 bp products (lanes 1–10) . Lane M: 1kb ladder.

Table 4. Accession number and identity of selected strain of *Yersinia enterocolitica* with GenBank

Strains of study	Match accession No.	Identity (%)
DB 30 *Yersinia enterocolitica*	JX424036.1	100%
DB 34 *Yersinia enterocolitica*	Z49828.1	100%
DB 37 *Yersinia enterocolitica*	LC0609151	100%

All strains were subjected to PCR using 16S rRNA. The PCR results in all strains showed a band size of 1,500 bp (Fig. 1). Three *Yersinia enterocolitica* strains were selected randomly for DNA sequencing. The results of sequencing and related accession numbers in the GenBank are listed in Table 4.

Multiple sequence alignment was done for three strains in the present study (DB30, DB34 and DB37) with two GenBank sequences (JX855135.1 and JX424036.1). Results showed that there was one change between the sequences at the locus 764 (Fig. 2). The phylogenic tree of the results showed that the local isolates were closely related to those strains previously registered from China (Fig. 3).

Virulence genes

The result of polymerase chain reaction for invasion (*inv*) gene, *Yersinia* adhesion A (*yad*A) and attachment invasion locus (*ail*) virulence genes showed that all *Y. enterocolitica* strains had the *inv* gene (100%) in their genome. In contrast, the *ail* gene was found in one strain only (10%) while the *yad* gene appeared in 50% of the investigated strains (Table 5).

DISCUSSION

Milk is a complete food, especially for children and seniors due to high content of proteins, minerals, fats, and vitamins. It is the primary source of nutrients for young mammals before they can digest other types of foods. That is why milk contamination is an important source for infections and illness (Rahimi, 2014).

Yersinia spp. especially *Y. enterocolitica* comprised strains with different degree of pathogenicity. In 2007, there were 8,792 reported cases of human yersiniosis in the European Union, making *Yersinia* the third most important zoonotic agent implicated in human enteritis, in terms of the number of cases, after *Campylobacter* and *Salmonella* (EFSA-ECDC, 2012; Tan *et al*., 2014). *Y. enterocolitica* has a spe-

```
JX855135.1 ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGAC 780
DB30       ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAGACTCAAATGAATTGAC 780
DB34       ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAGACTCAAATGAATTGAC 780
DB37       ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAGACTCAAATGAATTGAC 780
JX424036.1 ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAGACTCAAATGAATTGAC 780
           ****************************************** ***************
```

Fig. 2. Alignment sequences of three *Yersinia enterocolitica* isolates (DB30, DB34, DB37) along with two previously GenBank registered bacteria.

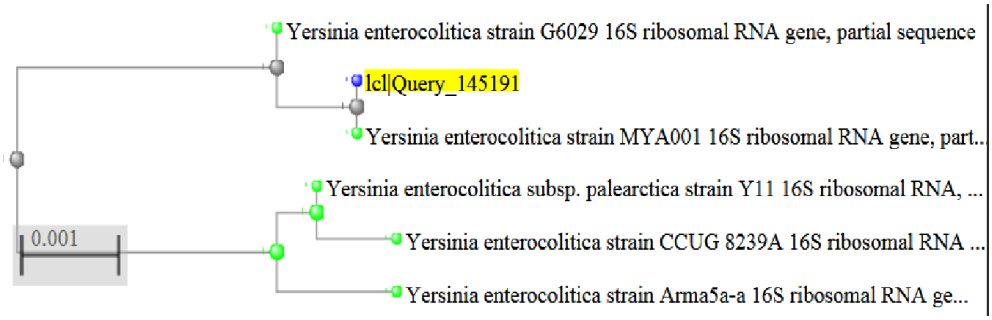

Fig. 3. Phylogenic neighbouring tree of local isolate of *Yersinia enterocolitica* with other GenBank registered isolates.

Table 5 . Presence of virulence genes in *Y. enterocolitica*

Gene	Total strains	Positive result	Percentage %
Inv	10	10	100%
Yad	10	5	50%
Ail	10	1	10%

cial public health importance, because of its capability of growing in raw milk and viability at refrigeration temperatures for a long time. Therefore, the consumption of milk and dairy products especially raw ones gives a higher chance for infection with *Y. enterocolitica* in humans (Rahimi *et al*., 2014).

In this study all bacteria recovered after cold enrichment for 21 days and after TSB enrichment for overnight incubation at 30 ^{o}C. The psychrotrophic nature of *Y. enterocolitica* is unusual among other Enterobacteriaceae. Consequently, enrichment in different solutions at 4 °C for prolonged periods is used for isolation of *Yersinia* spp. However, the time needed for this method is a disadvantage for routine analysis. In buffalo milk 22% and 8% were enriched by TSB and PBS. Both results were higher than those of Gamal Eldin (2008) – 7% and Khairalla (2006) – 6%. In cow milk results were lower than rate of 39.1%, detected by Askr *et al.* (2013) and higher than that detected by Ruusunen *et al.* (2013) and El-Prince & Elsyad (2002). The prevalence in sheep milk was 12% but *Y. enterocolitica* were not isolated by El-Leboudy & Gamel (1994). In a study in Morocco (Hamama *et al*., 1992), *Yersinia* spp. was recovered from 11 of 30 raw milk (36.6%), 1 of 20 pasteurised milk (5%), 15 of 63 traditional fermented milk (23.8%), 7 of 94 cheeses (7.44%), and 1 of 20 cream samples (5%). The overall incidence of *Y. enterocolitica* in milk and dairy products at previous study was 6.6%, which was similar to our results (6.66%). Another study showed that the prevalence of *Y. enterocolitica* was 24.1% in raw buffalo milk; however, no isolation was made from the pasteurised milk samples (Toora *et al.*, 1989). Sheep usually have a mass of fat attached to the back of their rump and it is in touch with faecal contamination. Thus sheep milk is likely to be contaminated by faeces during milking. This contamination is different than that observed in the survey previously conducted in Iran and other countries on several kinds of dairy products. In Turkey, 47.3% prevalence of *Y. enterocolitica* contamination was observed (Yucel & Ulusoy , 2006). In Mexico, Bernardino-Varo *et al*., (2012) reported a 34.92% prevalence of *Y. enterocolitica* contamination. In another study 9 sheep milk samples (9%) were positive for *Y. enterocolitica*. In Iran, (Jamali *et al.,* 2015) showed that *Y. enterocolitica* contamination of sheep milk samples was 3% and that of goat milk samples was 2.4%.

Virulence genes, such as *ail* and *yst*, are located on the bacterial chromosome. The Ail protein is encoded by the *ail* gene and occurs only in pathogenic *Y. enterocolitica*; it helps the bacterial adhesion to the host cell as well as intensifies resistance to the bactericidal effects of complement (Hanifian & Khani, 2012; Jamali *et al.,* 2015). In this study the result of presence of virulence genes differ from research data obtained by Saberianpour *et al.* (2014). In that study, multiplex PCR assay results showed that chromosomal virulence genes included *inv* (100%), *ail* (50%) and *ystA* (51.85%), and plasmid-encoded virulence factors included *yadA* (44.74%) and *virF* (35.18%). Those results agree with prevalence of *inv* gene in the present study (100%), and was lower than prevalence of *yadA* (26%). In another study, the *ail* gene was found in 100% of pathogenic *Y. enterocolitica* strains and the *yadA* gene – in only 86% of pathogenic *Y. enterocolitica* strains, but neither of the genes were detected in nonpathogenic strains of *Yersinia* spp. (Blais & Phillippe, 1995). In a previous research

multiplex PCR test results showed the *ail* gene found in 4 (4%) isolates, the *yadA* factor in 3 (3%), the *virF* and *ystA* genes in 2 (2%) of *Y. enterocolitica* strains while in present study, *Inv* gene found in 100% of the strains, *YadA* in 50%, and *ail* gene in 10% of the investigated strains. However, the *ail* gene was only detected from biotype 4/O:3 and all isolates of *Y. enterocolitica* biotype 1A only harboured the *yst*B gene and the *ystA* gene was not observed in all isolates. Hanifian & Khani (2012) reported 2.26% isolation of the *ail* gene of raw milk and cheese samples.

In this study all tested isolates were susceptible to streptomycin, azithromycin and gentamycin, 93.3% – to ciprofloxacin and chloramphenicol. Only fewe were susceptible to vancomycin (6.66%) and cloxacillin (33.3%). These rates differ from results of Soltan-Dallal *et al.* (2004) who reported that all samples were resistant to ampicillin and sensitive to tetracycline. In another study in Sardinia, all isolates were susceptible to cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, sulphonamide, tetracycline and trimethoprim-sulphametoxazole. Resistances to ampicillin and cefalothin were the most common (100%), followed by amoxicillin/clavulanic acid (83.0%) and streptomycin (4.3%).

CONCLUSION

On the basis of presented results, we conclude that the higher occurrence of *Yersinia enterocolitica* in cow milk, cow cheese samples in comparison with buffaloes milk, buffaloes cheese, sheep milk and sheep cheese samples. The isolated bacteria containing virulence genes and enterotoxin gene that helps in pathogenicity of these bacteria which showed resistance toward some commonly used antibiotics.

REFERENCES

Abbas, B. A. & A. B. Aldeewan, 2009. Occurrence and epidemiology of *Brucella* spp. in raw milk samples at Basrah province, Iraq. *Bulgarian Journal of Veterinary Medicine*, **12**, 136−142.

Abbas, B. A. & A. B. Talei, 2010. Isolation, identification and biotyping of *Brucella* spp. from milk product at Basrah province. *Basrah Journal of Veterinary Research*, **9**, 152–162.

Abbas, B. A. & G. M. Jaber, 2012. Occurrence of *Listeria monocytogens* in raw milk of ruminants in Basrah province. *Iraqi Journal of Veterinary Sciences,* **26**, 47–51.

Abbas, B. A., M. H. Khudor & O. I. Abid Smeasem, 2012a. Detection of verotoxigenic *E. coli* O157:H7 in raw milk using duplex PCR in Basrah city – Iraq. *Mirror of Research in Veterinary Sciences and Animals*, **1**, 25–33.

Abbas, B. A. M. H. Khudor & B. M. S. Saeed, 2012b. Molecular detection of *Bacillus cereus* emetic toxin gene by PCR and determine its susceptibility against *Punica granatum* extracts. *Basrah Journal of Veterinary Research*, **11**, 79–94.

Abbas, B. A., 2013. Detection of virulence and adherence gene in *Escherichia coli* O157:H7 isolated from animal products. *Basrah Journal of Veterinary Research*, **12**, 95–103.

Abbas, B. A., M. H. Khudor & O. I. Abid Smeasem, 2013a. Detection of *vt1* and *vt2* genes in *E. coli* O157:H7 isolated from soft cheese in Basrah, Iraq using duplex `PCR. *Journal of University of Zakho*, **1**, 58–64.

Abbas, B. A., M. H. Khudor & H. I. Idbeis, 2013b. Investigation of the activity and pathogenecity of *Staphylococcus aureus* enterotoxin by ligated ileal loop assay in

rabbits. *Basrah Journal of Veterinary Research*, **12**, 104–112.

Abbas B. A., M. H. Khudor & B. M. Hanoon, 2014a. Isolation and identification of *Staphylococcus aureus* from bovine and the detection of its coagulase gene (*coa*) using polymerase chain reaction (PCR). *Scientific Research and Essays*, **9**, 864–868.

Abbas B. A., M. H. Khudor & B. M, S. Saeed, 2014b. Detection of Hbl, Nhe and Bcet toxin genes In *Bacillus cereus* isolates by multiplex PCR. *International Journal* of *Current Microbiology and Applied Sciences*, **3**, 1009–1016.

Abbas B. A., M. H. Khudor & B. M. Hanoon, 2016. The relationship between biotype, serotype, antibiotic susceptibility and *coa* gene polymorphism in *Staphylococcus aureus* isolated from bovine. In: *Proceedings of the 17th Scientific Congress, Faculty of Veterinary Medicine, Assiut University, Egypt*, p. 33.

Abbas, B. A., M. K. Ghadban & A. M. Alghanim, 2017. microbial evaluation of milk and milk products during a past two decades, in Basrah Southern Iraq, a review. *Annual Research & Review in Biology*, **14**, 1–8.

Askr, A. A., S. F. Abd El Aal & I. H. Amer, 2013. Prevalence of virulent *Yersinia enterocolitica* in subclinical mastitic cow milk in Sharkia Governorate, Egypt. *Life Science Journal,* **10**, 1285–1294.

Bauer, A. W., W. M. M. Kirby, J. C. Sherris & M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**, 493–496.

Bergann, T., J. Kleemann & D. Sohr, 1995. Model studies of psychrotrophia in *Yersinia enterocolitica*. *Journal of Veterinary Medicine. Series B*, **42**, 523–531.

Bernardino-Varo, L., E. I. Quiñones-Ramírez, F. J. Fernández & C. Vazquez-Salinas, 2013. Prevalence of *Yersinia enterocolitica* in raw cow's milk collected from stables of Mexico City. *Journal of Food Protection*, **76**, 694–698.

Bharathy, S., S. C. Swetha, R. Venkateswara, S. Sudhanthiramani & B. Radhika, 2014. Prevalence and antibiogram of *Yersinia enterocolitica* in milk and fecal swab of dairy product cows from different places of Tirupthi Region Andhra Pradesh, India. *International Journal of Recent Scientific Research,* **6**, 5469–5475.

Blais, B. W. & L. M. Phillippe, 1995. Comparative analysis of *yadA* and *ail* polymerase chain reaction methods for virulent *Yersinia enterocolitica*. *Food Control*, **6**, 211–214.

Cornelis, G. R., 1998. The *Yersinia* deadly kiss. *Journal of Bacteriology*, **180**, 5495–5504.

Eden, P. A., T. M. Schmidt, R. P. Blakemore & N. R. Pace, 1991. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *International Journal of Systematic and Evolutionary Microbiology*, **41**, 324–325.

EFSA-ECDC (European Food Safety Authority & European Center for Disease Prevention and Control), 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food borne outbreaks in 2010. *European Food Safety Authority Journal,* **10**, 1–442.

El-Leboudy, A. A. & F. Gamel, 1995. Occurrence of gram-negative bacteria in goat's and sheep's milk. In: *Proceedings of the 6th Scientific Congress, Assiut (Egypt),* 20–22 November 1994.

El-Prince, E. & A. S. Sayed, 2002. Prevalence of *Yersinia enterocolitica* in milk and faeces of some lacting animals and typing of the obtained isolates by plasmid profile. *Assiut Veterinary Medical Journal,* **46**, 54–71.

Feng, P., S. P. Keasler & W. E. Hill, 1992.Direct identification of *Yersinia enterocolitica* in blood by polymerase chain reaction amplification. *Transfusion*, **32**, 850–854.

Gamal El-Din, L., 2008. *Yersinia enterocolitica* isolates from buffalo's raw milk: With special reference to outer membrane protein profile. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

Greenwood, M. H. & W. L. Hooper, 1989. Improved methods for isolation of *Yersinia* species from milk and food. *Food Microbiology,* **41**, 99–104.

Hamama, A., A. El Marrakchi & F. El Othmani, 1992. Occurrence of *Yersinia enterocolitica* in milk and dairy products in Morocco. *International Journal of Food Microbiology*, **16**, 69–77.

Hanifian, S. & S. Khani, 2012. Prevalence of virulent *Yersinia enterocolitica* in bulk raw milk and retail cheese in northern-west of Iran. *International Journal of Food Microbiology,***155**, 85–92.

Jamali, H., M. Paydar, B. Radmehr & S. Ismail, 2015. Prevalence, characterization, and antimicrobial resistance of *Yersinia* species and *Yersinia enterocolitica* isolated from raw milk in farm bulk tanks. *Journal of Dairy Science*, **98**, 798–803.

Jourdan, A. D., S. C. Johnson & I. V. Wesley, 2000. Development of a fluorogenic 5′ nuclease PCR assay for detection of the ail gene of pathogenic *Yersinia enterocolitica*. *Applied and Environmental Microbiol*ogy, **66**, 3750–3755.

Kapperud, G., T. Vardund, E. Skjerve, E. Hornes & T. E. Michaelsen, 1993. Detection of pathogenic *Yersinia enterocolitica* in foods and water by immunomagnetic separation, nested polymerase chain reactions, and colorimetric detection of amplified DNA. *Applied and Environmental Microbiol*ogy, **59**, 2938–2944.

Khairalla, H. M. A., 2006. Role of raw milk in transmission of some food poisoning organisms and parasites to consumer. Ph.D. thesis, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Khudor, M. H., B. A. Abbas & B. M. S. Saeed, 2012. Molecular detection of enterotoxin (*Cyt K*) gene and antimicrobial susceptibility of *Bacillus cereus* isolates from milk and milk products. *Basrah Journal of Veterinary Research*, **11**, 164–173.

Marshall, R. T., 1992. Standard Methods for the Examination of Dairy Products, 16th edn, American Public Health Association Washington, DC.

Mayrhofer, S., P. Paulsen, J. M. Smulders & F. Hilbert, 1992. Food borne pathogens isolated from beef, pork and poultry. *International Journal of Food Microbiology,* **97**, 23–29.

Miller, V., & S. Falkow, 1988. Evidence for two genetic loci in *Yersinia enterocolitica* that can promote invasion of epithelial cells. *Infection and Immunity*, **56**, 1242–1248.

Rahimi, E., S. Sepehri, F. S. Dehkordi, S. Shaygan & H. Momtaz, 2014. Prevalence of *Yersinia* species in traditional and commercial dairy products in Isfahan Province, *Iran. Jundishapur Journal of Microbiology*, **7**, e9249.

Ruusunen, M., M. Salonen, H. Pulkkinen, M. Huuskonen, S. Hellström, J. Revez, M. L. Hänninen, M. Fredriksson-Ahomaa, & M. Lindström, 2013. Pathogenic bacteria in finnish bulk tank milk. *Foodborne Pathogens* and *Disease,* **10**, 99–106.

Saberianpour, S., E. Tajbakhsh, & F. Khamesipour, 2014. Prevalence of virulence genes and biotyping of *Yersinia enterocolitica* isolated from chicken meat in Shahrekord, Iran. *Vidyabharati International Interdisciplinary Research Journal*, **3**, 71–76.

Sadek, O. A., S. M. Sayed, S. M. El Berbawy, M. F. Mansy & M.F. Hussien, 2014. Some antibiotic resistant bacteria of public health hazards isolated from raw milk sold in some Assiut city markets. *Assiut University Bulletin for Environmental Researches*, **17**, 97–107.

Saeed, B. M. S., B. A. Abbas, & S. A. N. Aljadaan, 2018. Molecular detection of tetracycline resistance genes in *Bacillus cereus*

isolated from food sources. *Basrah Journal of Veterinary Research,* **17**, 223–234.

Schulte, S., S. Kerneis, S. Klinke, H. Bartels, S. Preger, J. P. Kraehenbuhl, E. Pringault & I. B. Autenrieth, 2000. Translocation of *Yersinia enterocolitica* across reconstituted intestinal epithelial monolayers is triggered by *Yersinia* invasion binding to b1 integrins apically expressed on M-like cells. *Cellular Microbiology*, **2**, 173–185.

Selma, M. V., D. Beltrán, E. Chacón-Vera, & M. I. Gil, 2006. Effect of ozone on the inactivation of *Yersinia enterocolitica* and the reduction of natural flora on potatoes. *Journal of Food Protection,* **69**, 2363–2357.

Soltan-Dallal, M. M., A. Tabarraie, & K. MoezArdalan, 2004. Comparison of four methods for isolation of *Yersinia enterocolitica* from raw and pasteurized milk from northern Iran. *International Journal of Food Microbiology*, **94**, 87–91.

Tan, L. K., P. T. Ooi, & K. L. Thong, 2014. Prevalence of *Yersinia enterocolitica* from food and pigs in selected states of Malaysia. *Food Control,* **35**, 94–100.

Thoerner, P., C. I. Bin Kingombe, K. Bögli-Stuber, B. Bissig-Choisat, T. M. Wassenaar, J. Frey & T. Jemmi, 2003. PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. *Applied and Environmental Microbiol*ogy, **69**, 1810–1816.

Toora, S., G. Singh, R. P. Tiwari, & G. Singh, 1989. Drug resistance and lecithinase activity of *Yersinia enterocolitica* isolated from buffalo milk. *International Journal of Food Microbiology*, **9**, 167–171.

WHO, 2007. The World Health Report - A Safer Future Global Public Health Security in the 21st Century. https://www.who.int/whr/ 2007/whr07_en.pdf?ua=1 (5 July 2019 date last accessed).

Yucel, N. & H. Ulusoy, 2006. A Turkey survey of hygiene indicator bacteria and *Yersinia enterocolitica* in raw milk and cheese samples. *Food Control Journal*, **17**, 383–388.

Paper received 25.03.2019; accepted for publication 29.06.2019

Correspondence:

Basil A. Abbas
College of Veterinary Medicine,
University of Basrah, Iraq,
e-mail: basilabbas63@uobasrah.edu.iq