International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(11): 129-137
www.biochemjournal.com
Received: 23-08-2025
Accepted: 27-09-2025

M Venkata Lakshm
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

R Annie Supriya
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Garividi, Andhra Pradesh, India

P Ramya
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

B Sreedevi A
Department of Veterinary Microbiology, College of veterinary science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

C Siva Swetha
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Sohail Ahmed
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

P Vinayaka Siddartha
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

N Praharshini
Department of Veterinary Public Health and Epidemiology, College of veterinary science, Sri Venkateswara Veterinary University, Garividi, Andhra Pradesh, India

Corresponding Author:
R Annie Supriya
Department of Veterinary Public Health and Epidemiology, College of Veterinary science, Sri Venkateswara Veterinary University, Garividi, Andhra Pradesh, India

Molecular characterization of *yersinia enterocolitica* isolated from different parts of pig carcasses

M Venkata Lakshmi, R Annie Supriya, P Ramya, B Sreedevi A, C Siva Swetha, Sohail Ahmed, P Vinayaka Siddartha and N Praharshini

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i11b.6186

Abstract
*Yersinia enterocolitic*a is an emerging foodborne pathogen having worldwide public health concern. The present study was undertaken to characterize *Yersinia enterocolitica* from porcine origin using conventional and molecular methods, virulence gene profile and antibiogram. A total number of 302 samples from different parts of pig carcasses were procured from different areas in and around Tirupati, Andhra Pradesh which includes farms, retail shops and home butcheringand 44 hand swabs from personnel engaged in slaughter. Isolation and identification of *Yersinia enterocolitica* was done by conventional methods. The molecular characterization of *Yersinia enterocolitica* and gene profiling was performed using PCR and antibiogram by Kirby-Bauer method.

The highest prevalence was observed in farms (32%) followed by home butchered pigs (21%) and retail meat shops (7.6%). Whereas, thigh muscle, lung, tongue, mesenteric lymph nodes and hand swabs has shown 27% and 16% in neck muscle. Out of 76 PCR confirmed *Yersinia enterocolitica* isolates, the percentage positivity for*ail* gene was (7.8%),*Yst A*gene (14.4%) and *Yad A* gene (7.8%). All the three virulence genes were found in 30.3% of isolates. Antibiogram against 10 different antibiotics has shown maximum resistance to Azithromycin (98%), and the maximum sensitivity to Gentamicin (98%).

Keywords: *Yersinia enterocolitica*, food borne pathogen, pig carcass, hand swabs and public health significance

Introduction
Food borne zoonotic illnesses are one of the major concerns in today's lifestyle. The main causes of these food borne illness are unhygienic practices in food production, harvesting, and preparation (Adley *et al*., 2016) [1]. Foodborne pathogens can be detected at several points in the supply chain and determining the source of where these pathogens arise, their behavior throughout meat production and processing are important parts of risk-based approaches (Fegan *et al*., 2018) [12].

Pork high in protein and a versatile meat making it an ideal choice for non-vegetarian diet in different cuisines. The meat can act as a source of contamination for various pathogenic organisms. *Yersinia enterocolitica* is ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods. It is facultative, anaerobic, non-lactose fermenting gram-negative bacilli. Although the routes of human infection remain largely unresolved, contact with infected individuals or animals and intake of contaminated water or food are regarded as the most likely sources. *Yersinia enterocolitica* survive for long periods in the environment and remains metabolically active at extreme temperatures. These factors contribute to its transmission to humans by the fecal-oral route (Subha *et al*., 2009) [39].

Human yersiniosis is attributed to contaminated pork, milk, water as well as blood transfusion. Pig is a sentinel animal for *Yersinia enterocolitica* which causes Yersiniosis. *Yersinia enterocolitica* has caused high rate of morbidity and mortality, globally among children as a result of poor hygiene and lack of access to potable drinking water. Diarrheal diseases are major cause of children morbidity and mortality worldwide especially in developing countries.

Pigs are reported to be a major source of *Yersinia enterocolitica* and harbor the organism in their throat and tonsils as well as shed the organisms in feces. Raw meat of infected animals can become contaminated during slaughtering. During the time of slaughter, spillage of intestinal contents and tonsillar contents on to other body parts can lead to further spread of infection. If good hand hygiene is not practiced after using the toilet or handling raw meat, a person with *Yersinia* bacteria can transfer the bacteria to food and objects.

Yersinia enterocolitica can be isolated by conventional methods (Bharathy *et al*., 2014) [6]. Molecular confirmation of *Yersinia enterocolitica* can be done by PCR assay using organism specific primers (Ghada *et al*., 2017) [19]. *Yersinia enterocolitica* can be identified by biochemical tests such as citrate, methylred, esculin, triple sugar iron, urease, motility at 25 °C and 37 °C, H2S production, indole production, Voges-Proskauer and citrate utilization. PCR assays and other molecular methods have been developed as efficient tools for identifying pathogenic *Yersinia enterocolitica* and targeting chromosomal genes such as *ail* (attachment invasion locus which mediates cell invasion) (Miller *et al*., 1989)) [29], as well as plasmid gene *yad A* (whose product is involved in autoagglutination, serum resistance and adhesion) (Cornelis *et al*., 1989) [9] and another chromosomal genes *ystA* (which is responsible for the production of a heat-stable enterotoxin in virulent *Yersinia enterocolitica*) (Delor *et al*., 1990)) [10].

Yersinosis can be prevented by proper personal hygiene, handling the carcass in clean environment, by proper precautions during slaughtering process like avoiding spillage during evisceration and decapitation of the head to avoid spread of infection from tonsils to surrounding parts, thorough washing of hands and fingernails after handling raw pork and avoiding eating raw or under cooked pork.

Materials and methods

The present work was carried in the Department of Veterinary Public Health and Epidemiology and Veterinary Microbiology, College of Veterinary Science, Tirupati.

A total number of 302 samples were collected from different parts of pig carcasses and hand swabs of the butchers which were collected from different places of Tirupati like pig farms located in Renigunta(40), AICRP on Piggery, College of Veterinary Science, Tirupati (90), Pathamangalam (25), Appalayagunta (15) and from home butchered pigs a total of 80 samples were collected which includes Mangapuram (31), Tatithopu (34) and Tiruchanurroad (15). Further a total of 52 samples were collected from retail meat shop in Balaji colony, Tirupati Fig-1.0 andTable-1.0

Table 1: Number of samples analyzed for the study

S. No	Sampling area	Sampling site	Type of sample								Total
			M	T	L	I	N	NM	TM	H	
1.	Farms	R	8	8	8	8	8	-	-	-	40
2.		S	18	11	12	8	10	4	5	22	90
3.		P	5	5	5	5	5	-	-	-	25
4.		A	3	3	3	3	3	-	-	-	15
5.	Home butchered pigs	Ti	3	3	3	3	3	-	-	-	15
6.		M	5	5	5	4	2	2	3	5	31
7.		T	6	5	6	5	1	1	2	8	34
8.	Retail meat shops	B	8	7	7	7	5	5	4	9	52
			56	47	49	43	37	12	14	44	302

M-Thigh Muscle, T-Tongue, L-Lung, I- Intestinal Contents, N- Mesenteric Lymph nodes, NM-Neck muscles, TM-Thoracic muscle, H-Hand swabs.

R-Renigunta, S-AICRP on pigs, CVSc, Tirupati, P-Pathamangalam, A-Appalayagunta, Ti- Tiruchanur, M-Mangapuram, T-Tatithopu, B-Balaji colony.

The method for isolation and identification of the *Yersinia enterocolitica* was carried out using conventional culture method and biochemical tests for confirmation as described by (Baghel and Kumar, 2017); (Hudson *et al*., 2008); (Arora *et al*., 2012) and (Subha *et al*., 2009) [5, 20, 4, 39]. The biochemically confirmed colonies of *Yersinia enterocolitica* were detected by species specific PCR as described by (Wannet *et al*., 2001) [49]. All the biochemically confirmed *Yersinia enterocolitica* isolates from different sources were screened for the presence of virulence genes (*ail*, *ystA*, *yadA*) by multiplex PCR (m-PCR) as described by (Momtaz *et al*., 2013) [30]. PCR products were subjected to 1.5% agarose gel electrophoresis.

Table 2: List of oligonucleotide primers used in this study

Name of the primer	Pathogen	Primer Sequence	Amplicon length	Specific function of the gene	Reference
16SrRNA- F	Y.E	5'-AATACCGCATAACGTCTT CG-3'	330bp	Virulent marker- Adhesion A	(Wannet *et al*., 2001) [49]
16SrRNA-R		5'-CTTCTTCTGCGAGTAACG TC-3')			
YadA-F	Y.E. V	5'-CTTCAGATACTGGTGTCG CTG T- 3'	849bp	Virulent marker for attachment invasion locus	(Momtaz *et al*., 2013) [30]
YadA-R		5'-ATGCCTGACTAGAGCGAT ATC C - 3'			
ail-F		5'-ACTCGATGATAACTGGGG AG- 3'	170bp	Virulent marker for pathogenicity	
ail-R		5'-CCCCCAGTAATCCATAAA GG - 3'			
YstA-F		5'-AATGCTGTCTTCATTTGG AGC A - 3'	145 bp	Virulent marker- Enterotoxin	
YstA-R		5'-ATCCCAATCACTACTGAC TTC - 3'			

All the samples positive for the presence of *Yersinia enterocolitica*by molecular method were tested by Disc Diffusion (DD) method to detect their antibiotic sensitivity pattern. This method was performed using Mueller Hinton agar (Hi Media Laboratories, Mumbai, India).

Results

On CIN (Cefsulodin Irgasan Novobiocin) agar the culture have shown colonies with deep red centers and an outer translucent border. The colonies were flat with smooth border and entire edge has given a characteristic "Bull's eye" (Fig-1.0) On Grams staining, pink colored coccobacillary rods were observed under compound microscope (Fig-2.0) which is characteristic of *Yersinia enterocolitica*. On stab culture using motility agar, a diffuse hazy growth observed which indicates the organism as motile. (Fig.3)

Fig 1: *Yersinia enterocolitica* on CIN agar

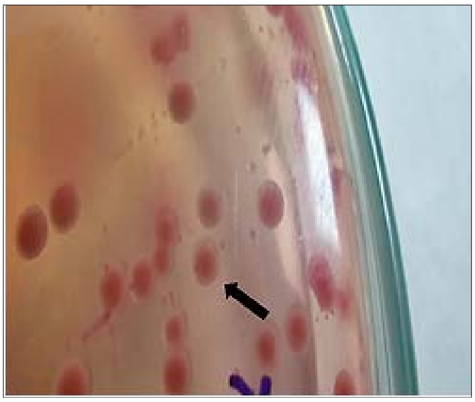


Fig 1 a: Colony morphology of *Yersinia enterocolitica*

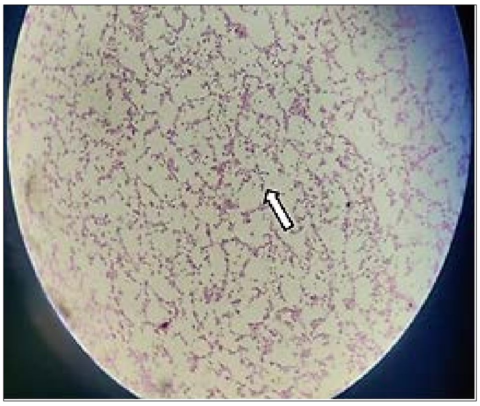


Fig 2: *Yersinia enterocolitica* on Gram staining

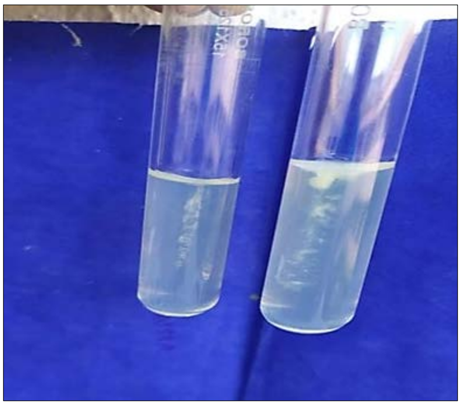
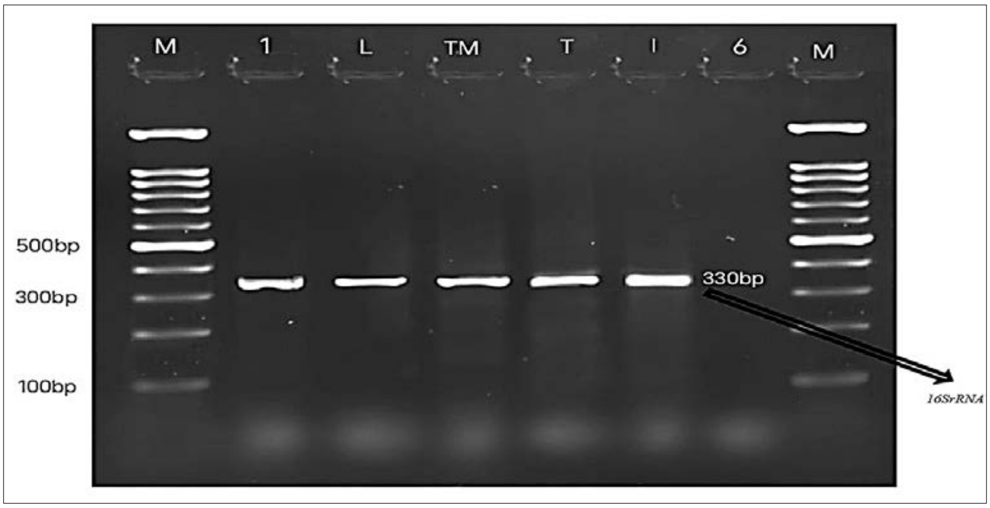


Fig 3: *Yersinia enterocolitica* on motility agar

All the presumptive positive samples of *Yersinia enterocolitica* were subjected to PCR by amplifying *16SrRNA*at 330bp length for confirmation of *Yersinia enterocolitica*. The resultsrevealed that out of 136 isolates targeted for *16SrRNA* gene, 76 isolates were confirmed as *Yersinia enterocolitica*. (Fig.4)



Lane M: DNA ladder (100bp) **Lane:** Positive control **Lane L:** Lung sample**, Lane TM:** Thigh muscle sample**, Lane T:** Tongue sample **Lane I:** Intestinal contents **Lane 6: Negative control Lane M:** DNA ladder (100bp)

Fig 4: Agarose gel electrophoresis for PCR product targeting *16SrRNA* gene for *Yersiniac enterocolitica*:

A multiplex PCR assays was standardized for the detection of three virulence genes in *Yersinia enterocolitica* (*ail*, *ystA*and *yadA*) (Fig-5) Optimum results were obtained using 25µL reaction mixture and a 25cycle PCR with annealing temperature of 60 °C was found to be optimum for the amplification of *ail*, *ystA*and*yadA*gene, with amplicon size of 170bp, 145bp and 849bp respectively.

The percentage positivity for virulence genes for different study areas was given in the Table-13 and Fig-10, and the percentage positivity for virulence genes for different parts of pig carcasses was given inFig.6. The gene*ystA*was detected in 11(14%) isolates, whereas, 6(7.8%) isolates were detected with both*ail and yad A*gene. Among the 76 isolates of *Yersinia enterocolitica*the virulence genes ail, *ystA*and*yadA*together in combination were detected in 5 isolates

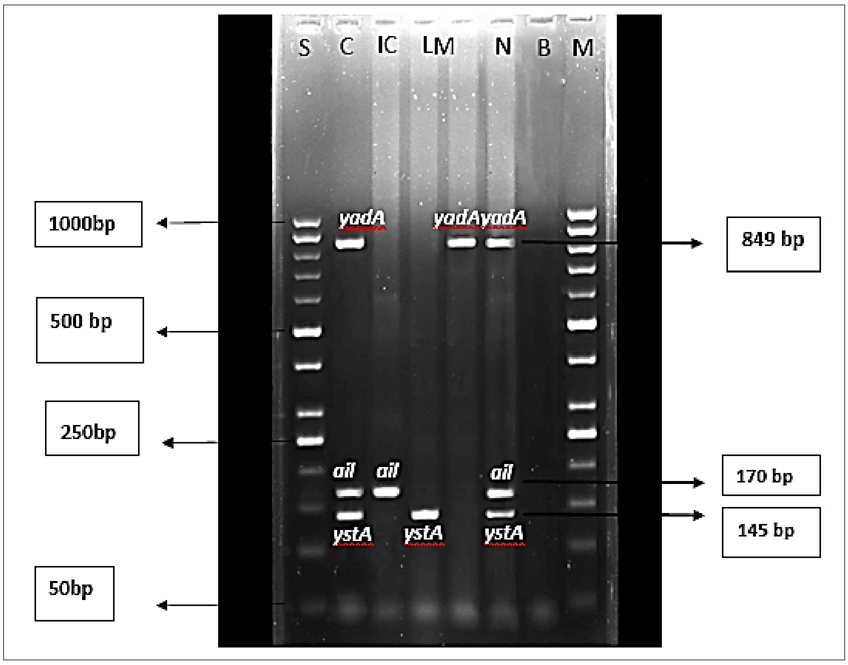


Fig 5: Agarose gel electrophoresis for PCR product targeting *ail* gene, *yst A* gene and *yad A* gene

Lane S: DNA ladder (50bp)
Lane C: Positive control of *Yersinia enterocolitica* (ATCC9610) showing *yst*Agene (145bp),
ailgene (170bp) and *yad* Agene (849bp).
Lane IC: *Yersinia enterocolitica* isolate from intestinal contents with *ail* gene (170bp)
Lane L: *Yersinia enterocolitica* isolate from lung sample with*yst A* gene (145bp)
Lane M: Yersinia *enterocolitica* isolate from thigh muscle with *yad A* gene (849bp)
Lane N: *Yersinia enterocolitica* isolate from mesenteric lymph node,*ystA* and *yadA* gene
Lane B: Negative control
Lane S: DNA ladde (50bp)

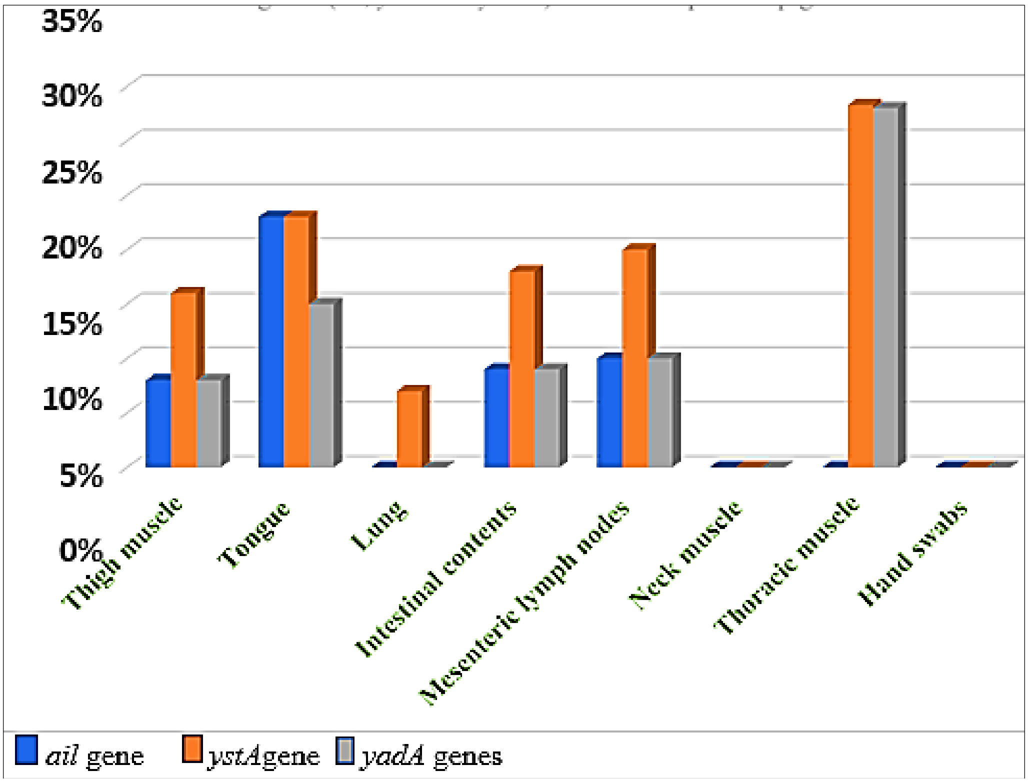
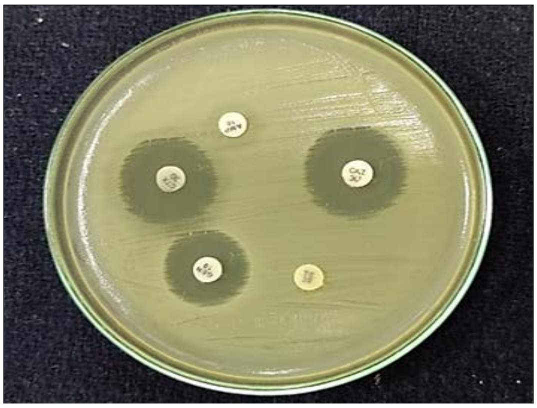


Fig 6: Graph depicting the prevalence levels of *Yersinia enterocolitica* for virulent genes (*ail*, *ystA and yadA*) in different parts of pig carcasses

All the 76 isolates of *Yersinia enterocolitica* were subjected to antibiotic sensitivity test using 10 different antibiotics Fig-7.0 and Fig-7.1.Among these, the isolates have shown maximum resistance to azithromycin (98%), ampicillin (94%) followed by cefotaxime (47%), ciprofloxacin (44%), ceftriaxone (21%), trimethoprim/sulfamethoxazole (2%), chloramphenicol(1%,) and there was no resistance to ceftazidime (0%), gentamicin (0%) and tetracycline (0%).The isolateshave shown maximum sensitivity to gentamicin (98%), trimethoprim/sulfamethoxazole (93%), tetracycline (92%),ceftazidime (89%), chloramphenicol (81%), followed by ceftriaxone (39%),cefotaxime(18%), ciprofloxacin(14%). Fig-8.0.



Ampicillin (10µg)- Resistant Gentamycin (10µg)- Sensitive Cefotaxime(30µg)-Intermediate Ceftazidime (30 µg)- Sensitive Tetracycline (30 µg)- Resistant

Fig 7: Plate showing antibiogram of *Yersinia enterocolitica* for different antibiotics tested in the study

Ceftriaxone (30µg)- Sensitive Ciprofloxacin(10µg)- Intermediate Chloramphenicol(30µg)-Intermediate Azithromycin (15µg)- resistant Trimethoprim/sulfamethoxazole(1.25/23.75)- Sensitive

Fig7.1: Plate showing antibiogram of *Yersinia enterocolitica* for different antibiotics tested in the study

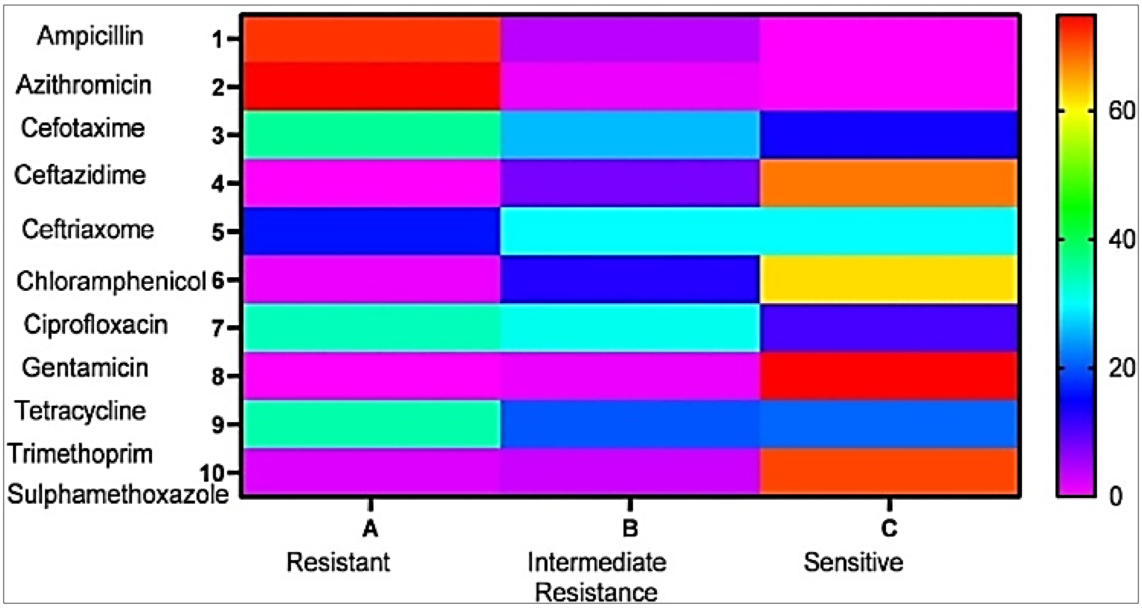


Fig 8: Heat map showing antimicrobial resistance patterns of *Yersinia enterocolitica* in the study

Discussion

Pork and pork products are excellent source of protein, vitamins, and minerals, but the production environments are very conducive to the growth of harmful bacteria, making them possible carriers of foodborne pathogens. Among several pathogens responsible for foodborne illness, *Yersinia enterocolitica* causes food infections predominantly by causing acute enteritis particularly in children (Drummond *et al*. 2012) [11].

Application of phenotypic methods were essential for the identification of *Yersinia enterocolitica*. CIN agar (Cefsulodin Irgasan novobiocin) was found to be relatively efficient for the isolation of *Yersinia enterocolitica* (Baghel and Kumar, 2017); (Tan *et al*., 2014) and (Ahmed *et al*., 2019) [5, 40, 2]. CIN agar (Cefsulodin, Irgasan and Novobiocin) was used in the present study for isolation of *Yersinia enterocolitica*, which was used by earlier researchers, for the recovery of *Yersinia enterocolitica* (Johannessen *et al*., 2000); (Vazlerova and Steinhauserova, 2006); (Wang *et al*., 2009); (Messelhausser *et al*., 2011) and (Tan *et al*., 2014) [22, 44, 48, 28].

All the colonies have shown characteristic Bull's eye appearance with deep red centers and transparent margins which were confirmed as *Yersinia enterocolitica* by conventional methods, similar findings were reported by (Van Damme *et al*., 2013) [43]. In the present study out of 302 samples, 136 samples identification was 45%. (Ramirez *et al*., 2000) [35] reported similar percentage positivity rate with present study as 49% and 45%. Whereas, (Fredriksson-Ahomaa and Korkeala, 2003) [15] reported 80% positivity by conventional methods for *Yersinia enterocolitica* in Finland which was higher than the present study.

The results of this study by PCR amplifying the *16SrRNA* gene at 330bp length revealed that, out of 302 collected samples for identification of *Yersinia enterocolitica*, 76 (25%) isolates have shown the presence of *16SrRNA* gene as per (Neubauer *et al.*, 2000) [33]. Overall prevalence of *Yersinia enterocolitica* in the present study was 25% which was higher than the reports of (Johannessen *et al*., 2000) [22], who reported 17% of prevalence for *Yersinia enterocolitica* from slaughter houses in Norway.

In the current work the prevalence rate of *Yersinia enterocolitica* observed in farms was 32%. Lower prevalence rate of *Yersinia enterocolitica* (8%) in farms were reported by Boral *et al*. (2018) [7] from India. Higher prevalence reported by (Drummond *et al*., 2012) [11], that was 69% in England, 100% in Italy and Spain and 80% in Belgium. (Virtanen *et al.*, 2012) [45] mentioned that higher prevalence of *Yersinia enterocolitica* in pig farms by may be due to addition of industrial by products in feed, frequent use of antibiotics which may increase the resistance of this pathogen, contamination through fecal shedding.

In the present study the prevalence rate of *Yersinia enterocolitica* observed in home butchered pig samples was 21%. (Fredriksson-Ahomaa *et al*, 2004) [17] reported the occurrence of *Yersinia enterocolitica* from butchered shops in Munich area of Germany as 8% to 25% and these findings are almost in agreement with the current study. Whereas, prevalence rate of *Yersinia enterocolitica* observed in retail meat shops as 7.6 % which was found to be lower compared to the results of (Fredriksson-Ahomaa *et al*., 2001) [18] from Helsinki, Finland and (Baghel *et al*., 2017) [5] from Haryana, India who have shown the results as 41 % and 18%.

In the present study, an overall prevalence of *Yersinia enterocolitica* was 25% which was recorded from different parts of pig carcass. The prevalence rates in thigh muscle, lung, tongue, intestinal contents, mesenteric lymphnodes, neck muscles, thoracic muscle as well as hand swabs of pig handlers was 21%, 26%, 27%, 25%, 27%, 16%, 21%, and 27% respectively. Higher prevalence rates of 27% was found in tongue, mesenteric lymph nodes and hand swabs.

The prevalence rate of *Yersinia enterocolitica* in tongue samples observed in the present study was 27% which was similar to the findings of (Ramirez *et al*., 2000) [35] which is 23% in Mexico. Higher prevelance was recovered by (Vishnubhatla *et al*., 2001) [46] with 67% by PCR method and 47% by conventional method in USA. (Arora *et al*., 2012)(4) reported 13% prevalence in pig tongue samples in Hisar, India, that was lower than the present work.

The prevalence rate of *Yersinia enterocolitica* in mesenteric lymph nodes in the present study was 27% which was lower than the findings of (Boyapalle *et al*., 2001) [8] in mesenteric lymph nodes was 40% by PCR method in USA, which was higher than the present work. Prevalence rate of present study was higher than the reports of (Fois *et al*., 2018) [14] which was 2.4% in lymph nodes of finishing pigs and 2.8% in piglets. (Martins *et al*., 2018) [27] reported 2.2% *Yersinia enterocolitica* in lymph nodes from Brazilian pork production chain which was lower than the present study.

The prevalence rate of *Yersinia enterocolitica* in intestinal contents observed in the present study was 27% which was higher than the reports of (Nesbakken *et al*., 2003) [32] that is 11.7% in Norway, and also reported frequency of virulent *Yersinia* varied from 4.2% to 16.7%. (Liang *et al.*, 2012) [25] reported the prevalence rate of *Yersinia enterocolitica* from intestinal contents of pigs as 7.51% in pigs slaughtered in Chinese abattoirs reported by [25] which was lower than present work. (Ibanez *et al.*, (2016) [21] reported the prevalence of *Yersinia enterocolitica* in intestinal contents of pigs in fattening farms was 31.9% and farrowing-and-fattening farms was 52% of pigs in Finland which was higher than the present study.

Due to the ability of pigs to harbour *Yersinia enterocolitica* for extended periods of time without displaying any clinical symptoms, pork and pork products have been shown to contain high levels of contamination (Laukkanen-Ninios *et al*., 2014 and Moreira *et al*., 2019) [24, 31]. (Yang *et al*., 2013) [50] and (Latha *et al*.,(2017) [23] reported 1 % prevalence for *Yersinia enterocolitica* from pork samples in Korea and India which was lower than the present work (21%). Odoi *et al*. (2021) [34] in Japan and (Terentjeva *et al*., 2021) [41] in Latvia reported prevalence of *Yersinia enterocolitica* as 21% and 23%from pork samples which was almost similar with the present study.

The higher prevalence of *Yersinia enterocolitica* inside of the gastrointestinal contents may be due to the slaughter house workers accidentally cut into the viscera with their knives, the contents of the stomach, ileum, caecum, and colon also provide contamination hazards of *Yersinia enterocolitica*.

In present study, all 76 isolates of *Yersinia enterocolitica* were subjected to optimize multiplex PCR assay for detection of *ail*, *ystA* and *yadA* genes. Out of 76 isolates, 11 isolates showed presence of at least one virulence gene while 65 isolates harbored none of the virulence genes indicating them to be probably non-pathogenic. The assay showed presence of *ystA*, *ail* and *yadA* virulence genes at the rate of 14.47%, 7.8% and 7.8% respectively.

In recent years, antimicrobial resistance has become a significant concern for human health. WHO on several occasions has declared antimicrobial resistance as a serious threat to human health globally (Sahota *et al*., 2012) [36]. Antibiotic sensitivity pattern was investigated to address this problem and document the antimicrobial resistance status among the *Yersinia enterocolitica* isolates which were isolated from various parts of pig carcasses.

Majority of the isolates showed resistance to ampicillin (94%) and azithromycin (98%). Fois *et al*. (2018) reported 100% resistance towards ampicillin and (Zdolec *et al*., 2022) [52] reported 91.6% which was almost like the present study. (Bharathy *et al*., 2014) [6] reported the resistance percentage of 16.67% for ampicillin in food samples which were lower than the present work.

In contrary to the high resistance rates observed against azithromycin in present study (98%), (Stock *et al*., 2002) [38] reported that all macrolides except azithromycin was naturally resistant to *Yersinia*, but (Martin Pozo *et al*., 2014) [26] reported that azithromycin would be a useful antibiotic alternative to treat bacterial diarrhea due to *Yersinia* infection. The variation in resistance rates could be attributed to the choice of antibioticlocally and possible indiscriminate use in some areas which is often there as on for antibiotic resistance.

In present study, majority of isolates showed susceptibility to gentamicin, trimethoprim/sulfamethoxazole, ceftazidime, and chloramphenicol at the rates of 98%, 93%, 89.4% and 81%. (Ye *et al*., 2015) [51] reported 60% susceptibility towards chloramphenicol which was lower compared to current study. (Fois *et al*., 2018) [14] reported susceptibility towards Cefotaxime, ciprofloxacin gentamicin, trimethoprim/sulfamethoxazole, ceftazidime and chloramphenicol, which was similar with the current study. Our study has reported resistance against cefotaxime and ciprofloxacin, whereas, (Anju *et al*., 2014) [3] has reported intermediate resistant to the similar compounds. In contrary to our study, (Wang *et al*., 2021) [44] and (Fois *et al*., 2018) [14] reported that, tetracycline was highly susceptible to *Yersinia enterocolitica*. In contrary to our study where resistance towards tetracycline was reported as 46%, (Fredriksson-Ahomaa *et al*., 2010) [16] reported 1% resistance to tetracycline, for *Yersinia enterocolitica.*

Our study revealed that, pork samples from retail meat shops have lower prevalence rates for the pathogen studied, while compared to samples from piggery farms and home butchered samples. The hygienic conditions maintained at the retail shops like maintaining personal hygiene, cleaner cutting boards and equipment used for meat cutting, dipping of knives in hot boiling water and using traditional disinfection methods for carcass and cutting boards like applying turmeric before cutting the carcass may be responsible for the lower counts of *Yersinia enterocolitica*. It may also be due to slaughtering few numbers of animals per day, one after other based on the demand from the consumers, minimizing the possibility for cross contamination.

The higher prevalence rates observed in the farms may be due to slaughtering a greater number of animals per day, cutting animals in the same place and using same knives for cutting, pooling of meat and offal's from different animals before sale, wandering of stray animals in the slaughtering premises, slaughtering the animal near the gutters might have increased the chances of contamination from the slaughter environment and cross contamination.

The higher prevalence rates of *Yersinia enterocolitica* was observed in tongue samples and mesenteric lymph nodes. Earlier studies indicated that, pigs asymptomatically carry this organism in their oral cavity and pharynx (mainly tonsil and tongue), lymph nodes and intestine. *Yersinia enterocolitica* obligately feeds on lymphoid tissues which increases the chances of its presence in mesenteric lymph nodes. Higher prevalence was also observed in hand swabs collected from pig handlers and slaughter men which may be due poor personal hygiene, handling of offal's with bare hands and lack of provision for hand washing with soap.

Conclusion

The level of carcass contamination can be reduced with hygiene practices like maintaining clean premises, following methods like removing the head and bunging the rectum immediately after slaughter, separation of meat and offal's, proper cleaning and disinfection of meat cutting equipment and proper waste disposal. Further bringing awareness among the pig farmers, butchers and retail meat shops is also very important to reduce the contamination of the carcasses with *Yersinia enterocolitica*. The present study revealed that *Yersinia enterocolitica* isolates were shown more resistance towards different classes of antibiotics which may pose a public health treats in future.

References

1. Adley CC, Ryan MP. The nature and extent of food borne disease. In: *Antimicrobial Food Packaging*. Academic Press; 2016. p. 1-10.
2. Fegan N, Jenson I. The role of meat in food borne disease: Is there a coming revolution in risk assessment and management? Meat Sci. 2018;144:22-9.
3. Subha B, Ramakrishnan D, Suganthi V. Antimicrobial resistance pattern of selected *Yersinia enterocolitica* isolates from raw cow milk and pork samples of Namakkal District, Tamil Nadu, South India. Glob Environ Res. 2009;3(3):169-77.
4. Bharathy S, Swetha SC, Venkateswara R, Sudhanthiramani S, Radhika B. Prevalence and antibiogram of *Yersinia enterocolitica* in milk and fecal swab of dairy product cows from different places of Tirupati Region, Andhra Pradesh, India. Int J Recent Sci Res. 2014;6:5469-75.
5. Ghada A, Nehal AA, Shereen AY. Evaluation of using molecular biology and traditional methods for detection of *Yersinia enterocolitica* in raw chicken meat. Anim Health Res Rev. 2017;5(4):367-74.
6. Miller VL, Farmer 3rd JJ, Hill WE, Falkow S. The *ail* locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease. Infect Immun. 1989;57(1):121-31.
7. Cornelis G, Sluiters C, De Rouvroit CL, Michiels T. Homology between virF, the transcriptional activator of the *Yersinia* virulence regulon, and AraC, the *Escherichia coli* arabinose operon regulator. J Bacteriol. 1989;171(1):254-62.
8. Delor I, Kaeckenbeeck A, Wauters G, Cornelis GR. Nucleotide sequence of *yst*, the *Yersinia enterocolitica* gene encoding the heat-stable enterotoxin, and prevalence of the gene among pathogenic and non-

pathogenic *Yersiniae*. Infect Immun. 1990;58(9):2983-8.
9. Drummond N, Murphy BP, Ringwood T, Prentice MB, Buckley JF, Fanning S. *Yersinia enterocolitica*: a brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. Foodborne Pathog Dis. 2012;9(3):179-89.
10. Baghel SS, Kumar A. Occurrence of *Yersinia enterocolitica* in raw pork. Haryana Vet. 2017;56(1):83-6.
11. Tan LK, Ooi PT, Carniel E, Thong KL. Evaluation of a modified cefsulodin-irgasan-novobiocin agar for isolation of *Yersinia* spp. PLoS One. 2014;9(8):106329.
12. Ahmed HA, Taoun ABMB, Abou Elez RMM, Abd El-Hamid MI, Abd Ellatif SS. Prevalence of *Yersinia enterocolitica* in milk and dairy products and the effects of storage temperatures on survival and virulence gene expression. Int Dairy J. 2019;94:16-21.
13. Johannessen GS, Kapperud G, Kruse H. Occurrence of pathogenic *Yersinia enterocolitica* in Norwegian pork products determined by a PCR method and a traditional culturing method. Int J Food Microbiol. 2000;54(1):75-80.
14. Vazlerova M, Steinhauserova IVA. The comparison of the methods for the identification of pathogenic serotypes and biotypes of *Yersinia enterocolitica*: Microbiological methods and PCR. Czech J Food Sci. 2006;24(5):217-22.
15. Wang X, Cui Z, Jin D, Tang L, Xia S, Wang H. Distribution of pathogenic *Yersinia enterocolitica* in China. Eur J Clin Microbiol Infect Dis. 2009;28:1237-44.
16. Messelhäusser U, Kampf P, Colditz J, Bauer H, Schreiner H, Höller C, *et al*. Qualitative and quantitative detection of human pathogenic *Yersinia enterocolitica* in different food matrices at retail level in Bavaria. Foodborne Pathog Dis. 2011;8(1):39-44.
17. Van Damme I, Berkvens D, Botteldoorn N, Dierick K, Wits J, Pochet B, *et al*. Evaluation of the ISO10273:2003 method for the isolation of human pathogenic *Yersinia enterocolitica* from pig carcasses and minced meat. Food Microbiol. 2013;36(2):170-5.
18. Ramirez EIQ, Vázquez-Salinas C, Rodas-Suarez OR, Pedroche FF. Isolation of *Yersinia* from raw meat (pork and chicken) and precooked meat (porcine tongues and sausages) collected from commercial establishments in Mexico City. J Food Prot. 2000;63(4):542-4.
19. Fredriksson M, Korkeala H. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. Clin Microbiol. 2003;16:220-9.
20. Neubauer H, Aleksic S, Hensel A, Finke EJ, Meyer H. *Yersinia enterocolitica* 16S rRNA gene types belong to the same genospecies but form three homology groups. Int J Med Microbiol. 2000;290(1):61-6.
21. Johannessen GS, Kapperud G, Kruse H. Occurrence of pathogenic *Yersinia enterocolitica* in Norwegian pork products determined by a PCR method and a traditional culturing method. Int J Food Microbiol. 2000;54(1):75-80.
22. Boral R, Rathore RS, Paul S, Mishra AK, Patra G. Isolation, identification, assessment of pathogenicity and antibiotic sensitivity of *Yersinia enterocolitica* isolates from pigs. Indian J Anim Health. 2018;57(1):51-60.
23. Virtanen S, Korkeala H. Enteropathogenic *Yersinia* in pig production. World's Vet Med. 2012;199:298-305.
24. Fredriksson-Ahomaa M, Koch U, Klemm C, Bucher M, Stolle A. Different genotypes of *Yersinia enterocolitica* 4/O:3 strains widely distributed in butcher shops in the Munich area. Int J Food Microbiol. 2004;95(1):89-94.
25. Fredriksson-Ahomaa M, Hallanvuo S, Korte T, Siitonen A, Korkeala H. Correspondence of genotypes of sporadic *Yersinia enterocolitica* bioserotype 4/O:3 strains from human and porcine sources. Epidemiol Infect. 2001;127(1):37-47.
26. Vishnu Bhatla A. Application of deoxyribonucleic acid (DNA) based techniques for the detection of *Yersinia enterocolitica* and *Fusobacterium necrophorum*. Kansas State University; 2001.
27. Boyapalle S, Wesley IV, Hurd HS, Reddy PG. Comparison of culture, multiplex, and 5′ nuclease polymerase chain reaction assays for the rapid detection of *Yersinia enterocolitica* in swine and pork products. J Food Prot. 2001;64(9):1352-61.
28. Fois F, Piras F, Torpdahl M, Mazza R, Ladu D, Consolati SG, *et al*. Prevalence, bioserotyping and antibiotic resistance of pathogenic *Yersinia enterocolitica* detected in pigs at slaughter in Sardinia. Int J Food Microbiol. 2018;283:6-12.
29. Martins BTF, Botelho CV, Silva DAL, Lanna FGPA, Grossi JL, Campos-Galvão MEM, *et al*. *Yersinia enterocolitica* in a Brazilian pork production chain: tracking of contamination routes, virulence and antimicrobial resistance. Int J Food Microbiol. 2018;276:5-9.
30. Nesbakken T, Eckner K, Hoidal HK, Rotterud OJ. Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. Int J Food Microbiol. 2003;80(3):231-40.
31. Liang J, Wang X, Xiao Y, Cui Z, Xia S, Hao Q, *et al*. Prevalence of *Yersinia enterocolitica* in pigs slaughtered in Chinese abattoirs. Appl Environ Microbiol. 2012;78(8):2949-56.
32. Ibañez TR, Laukkanen-Ninios R, Hakkinen M, Johansson T, Vilar M, Korkeala H. Prevalence of pathogenic *Yersinia enterocolitica* in Finnish slaughter pigs. J Food Prot. 2016;79(4):677-81.
33. Laukkanen-Ninios R, Fredriksson-Ahomaa M, Maijala R, Korkeala H. High prevalence of pathogenic *Yersinia enterocolitica* in pig cheeks. Food Microbiol. 2014;43:50-2.
34. Moreira LM, Milan C, Gonçalves TG, Ebersol CN, De Lima HG, Timm CD. Contamination of pigs by *Yersinia enterocolitica* in the abattoir flowchart and its relation to the farm. Cienc Rural. 2019;49(8):20181040.
35. Yang YM, Son JW, Choi TS, Park M, Kim JY, Lee JH, Shin BW. A survey of the microbial contamination level in butcher's shops in Seoul, Korea. Korean J Vet Serv. 2013;36(3):203-8.
36. Latha C, Anu CJ, Ajaykumar VJ, Sunil B. Prevalence of *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium in meat and meat products using multiplex polymerase chain reaction. Vet World. 2017;10(8):927-31.

37. Odoi JO, Takayanagi S, Sugiyama M, Usui M, Tamura Y, Asai T. Prevalence of colistin-resistant bacteria among retail meats in Japan. J Food Saf. 2021;9(2):48-56.
38. Terentjeva M, Ķibilds J, Meistere I, Gradovska S, Alksne L, Streikiša M, Valciņa O. Virulence determinants and genetic diversity of *Yersinia* species isolated from retail meat. J Pathog. 2021;11(1):37-44.
39. Sahota P, Verma SK, Mahajan V, Singh JP. Epidemiology, virulence and public health significance of *Yersinia enterocolitica* in drinking water. Afr J Microbiol Res. 2012;6(30):5905-13.
40. Zdolec N, Kis M, Jankuloski D, Blagoevska K, Kazazić S, Pavlak M, Pazin I. Prevalence and persistence of multidrug-resistant *Yersinia enterocolitica* 4/O:3 in tonsils of slaughter pigs from different housing systems in Croatia. Foods. 2022;11(10):1459-67.
41. Stock I, Wiedemann B. Natural antibiotic susceptibility of *Enterobacter amnigenus*, *Enterobacter cancerogenus*, *Enterobacter gergoviae* and *Enterobacter sakazakii* strains. Clin Microbiol Infect. 2002;8(9):564-78.
42. Martín-Pozo A, Arana DM, Fuentes M, Alós JI. Susceptibility to azithromycin and other antibiotics in recent isolates of *Salmonella*, *Shigella* and *Yersinia*. Enferm Infecc Microbiol Clin. 2014;32:369-71.
43. Ye Q, Wu Q, Hu H, Zhang J, Huang H. Prevalence, antimicrobial resistance and genetic diversity of *Yersinia enterocolitica* isolated from retail frozen foods in China. FEMS Microbiol Lett. 2015;362(24):1-8.
44. Anju P, Latha C, Sunil B, Sethulekshmi C. Antimicrobial resistance profile of *Yersinia enterocolitica* and *Yersinia intermedia* isolates from retail pork. Int J Curr Microbiol Appl Sci. 2014;3(8):231-4.
45. Wang J, Liu M, Wang H, Wu Q, Ding Y, Xu T. Occurrence, molecular characterization, and antimicrobial susceptibility of *Yersinia enterocolitica* isolated from retail food samples in China. LWT. 2021;150:111876.
46. Fredriksson-Ahomaa M, Lindström M, Korkeala H. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In: Juneja VK, Sofos NJ, editors. *Pathogens and Toxins in Foods: Challenges and Interventions*. Washington (DC): ASM Press; 2010. p. 164-80.