ORIGINAL RESEARCH PAPER

Microbiology

IDENTIFICATION AND GENE DETECTION OF COMMON SALMONELLA SEROTYPES IN MEAT AND POULTRY PRODUCTS FROM EGYPTIAN MARKETS

KEY WORDS: Salmonella, meat and poultry products, antimicrobial agents and invA gene.

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ABSTRACT

This investigation aims to identify and screen Salmonella serotypes isolated from one hundred meat and poultry products collected from local Egyptian market in 2018-2019. Morphological and biochemical identification were applied. Salmonella strains were highly susceptible to Ciprofloxacin (77.7% and 81.8%), kanamycin (72.2% and 63.6%), Ampicillin (55.5% and 45.4%) and Norfloxacin (38.3% and 63.6%) for meat and poultry products respectively. Then PCR was carried out to detect invA gene in isolated Salmonella. It was found that 36% (18/50) and 22% (11/50) of meat and poultry samples were positive for Salmonella isolation. Based on invA sequence alignments data, Salmonella serotype which isolated from meat products were identified as Salmonella newland (12.5%), Salmonella enteritidis (77.7%) and Salmonella hessarek (9.8%). On the other hand, Salmonella serotypes which isolated from poultry products were identified as Salmonella typhimurium (22.8%), Salmonella enteritidis (65.4%) and Salmonella Kentucky (11.8 %). Our obtaining results add more highlights for hygienic measurements to decrease meat and poultry products contamination with pathogenic Salmonella.

INTRODUCTION

Food contamination considers the priority for those interested in public health. Existence of pathogenic bacteria in meat and poultry products is a great public health concern which may occurs during any stage of food processing and distribution chains. (Nagappa et al., 2007). Since the 1980's salmonellosis consider one of the most common infectious diseases for both humans and animals with high mortality (Chiu et al., 2010 and Rodrigue et al., 1990). Annually about 1.2 million patients were diagnoses in the USA as salmonellosis with average curing costs of $365 million. (Centers for Disease Control and Prevention, 2014). Salmonellosis considered as the second most common gastrointestinal infection; about 20.4 cases were confirmed per 100,000 individuals in 2011. (European Centre for Disease Prevention and Control, 2013). Many of Salmonella serotypes can be transferred to poultry and meat from different feedstuffs, breeding, wild birds, and animals. Furthermore, a major public health concern for microbiologist is the probability of transfer of antimicrobial resistant genes between different bacterial species, in the light of dramatic increase of this resistance; as a consequence of wide spread use of these drugs (Panisello et al., 2000).

Antimicrobials are a great tool for treatment and control of Salmonella infections. Early 1990s had been remarked with existence of multidrug-resistant Salmonella strains especially for - lactams and fluoroquinolones. Thus, identification of Salmonella serotypes distribution in animal-food products, and their antibiotic resistance patterns, are very important for maintaining safe commercial trade of animal food products.(Lai et al., 2014; Russell et al., 2014).

Amplification specific gene via PCR method considers a powerful tool in microbiological diagnostics (Malorny et al., 2003). Salmonella species was detected via amplified virulence chromosomal genes including; invA, invE, himAphoP in natural environmental, food and faucal samples (Jamshidi et al., 2009). Salmonella invA gene contains distinguishable sequences to this genus. Thus, applied as detectable PCR target with promising diagnostic applications (Rahn et al., 1992).

Based on previous finding, our present investigation was carried out to evaluate the microbial quality of meat and poultry products sold at various local markets retails. In this investigation, samples of meat and poultry products were screened for Salmonella, through morphological, biochemical methods. For each Salmonella isolate, invA specific gene was amplified, eluted, sequenced and alignment to identify Salmonella serotypes.

Materials and methods

Samples collection

One hundred of meat and poultry products were collected as represented samples from local market. Sterile polyethylene bags were applied for sample reservation. Samples were transported under cooling conditions for morphological, biochemical and molecular identifications. 300 ml of mlbuffered peptone water (BPW) (Oxoid Limited, Hampshire, England) was applied to homogenize 30 gram of each sample in Stomacher ® Lab-Blender Bags Standard 80 (Optika, Italy) using Stomacher ® 400 Circulator (Thermo Fisher, USA).

Preparation of samples

Selective enrichment method was applied via inoculation of 1ml of the incubated pre-enrichment homogenate to selenite cystine broth (SC) (Difco) medium as selective medium and incubated at 37°C for 24 h. Finally, XLD agar , MacConky's agar and Salmonella –Shigella agar (SS) (Oxoid) were streaked with loopful from the selective enrichment broth and incubated at 37°C for 24 h. Based on morphological characters, typical colonies of Salmonellae were examined. Biochemical features were studied via methyl red, catalase, oxidase, voges-prauskauer test, motility, indole production, sugar fermentation tests. Colonies that represent biochemical characters like Salmonella were transferred to nutrient agar slant (Himedia) and incubated at 37°C for 24 hrs.

Antimicrobial susceptibility screening

Kirby –Bauer disk diffusion method (Finegold and Martin 1982). Antibiotic disks of Kanamycin (30 µg), Ciprofloxacin (5 µg), Ampicillin (25 µg) and Norfloxacin (10 µg) were applied on Muller Hinton agar for antimicrobial susceptibility screening according to Sharma et al., (2016).

Screening Salmonella isolates genotyping via invA gene molecular marker Genomic bacterial DNA were purified via E.Z.N.A. ® Bacterial DNA Kit (D3350-01). PCR molecular marker technique was applied for Salmonella isolates. Based on the invA gene which responsible for Salmonella invasion, specific primers S139 and S141 (5´- GTG AAA TTA TCG CCA CGT TCG GGC AA - 3´) and (5´-TCA TCG CAC CGT CAA AGG AAC C - 3´) were applied (Rahnet al. 1992). Specific DNA was amplified through Gene Amp Polymerase Chain Reaction (Creacon, Thermo cycler, Holand) system cycler. PCR for amplified genomic DNA was carried out through DreamTaq Green PCR Master Mix (2X) K1081 Thermo Fisher, USA) was applied according to manufacturer protocol.. The reaction conditions as follow, an initial incubation at 94 °C for 60 seconds, followed by 35 cycles of denaturation at 94 °C for 60 seconds, annealing at 64 °C for 30 seconds and elongation at 72°C, followed by 7 minutes final extension period at 72°C (Karmi 2013). Gel documentation system (Geldoc-it, UVP, England) was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1). Specific DNA bands were eluted from agarose gel. Resultant PCR products were purified with Micro

spin filters and quantities spectrophotometrically. Sequence analysis was employed using the ABI PRISM® 3100 Genetic Analyzer (Micron-Corp. Korea).

Data analysis:
Aligned sequences were analyzed on NCBI website (http://www.ncbi.nlm.nih.gov/webcite) using BLAST to confirm their identity. The Genetic distances and MultiAlignments were computed by Pairwise Distance method using ClusteralW software analysis (www.ClusteralW.com). The nucleotide sequences were also compared with reference gene sequences available in the GenBank.

RESULTS AND DISCUSSION

Our obtaining results indicate that, 29 % of whole meat and poultry products were identified as positive Salmonella isolates. Meat products samples were superior for positive Salmonella isolates comparing with poultry products. As shown by table (1), 18 and 11 positive Salmonella isolates with 36 % and 22 % were recorded for meat and poultry products respectively. In accordance with our findings, many meat and poultry products were heavily contaminated with Salmonella sp Kinsella et al., 2009 and Malorny et al., (2003).

Table (1): Total number, Positive and Salmonella Positivity Percentages for meat and poultry products.

Products type	Total number of samples	Positive Salmonella isolates	Positivity Percentages
Meat products	50	18	36
Poultry products	50	11	22

Biochemical characterization considers easiest distinguishable methodology for the isolates identification. By contrary, the most accurate serotype only detected via molecular genetics methods (Sharma and Das 2016). In our investigation, 29 % of isolates characterized as Salmonella genus based on chemical characterization including negative oxidase, positive catalase, positive indole in tryptone broth, positive methyl red, negative Voges-Proskauer, urease negative and citrate positive tests and fermented glucose, fructose and maltose.

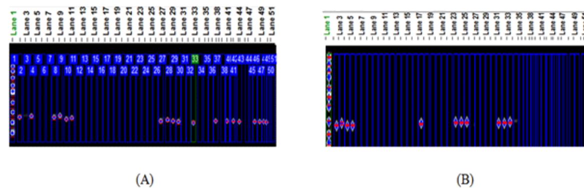
For our confirmed 29 Salmonella isolates, kanamycin, ciprofloxacin, ampicillin and norfloxacin antibiotics were tested for susceptibility. As shown by table (2), antimicrobial susceptibility patterns were studied against 4 antibiotics, ciprofloxacin was 77.7% and 81.8% susceptible, whereas kanamycin exhibited 72.2% and 63.6% susceptibility, Ampicillin showed 55.5% and 45.4%; while Norfloxacin showed 38.3% and 63.6%; for meat and poultry products respectively. More support was added to our findings of applied antimicrobial susceptibility patterns to identify Salmonella isolate patterns by Sharma and Das (2016).They found that, ciprofloxacin and kanamycin reflected the highest antimicrobial susceptibility percentage among varied applicable antibiotics. Additional antibiotics were added to identify Salmonella isolate susceptibility patterns by Mijovic1 et al., 2012.In accordance with our findings, Kebede et al., (2016) employed antibiotic susceptibility patterns to characterize Salmonella isolate infection in slaughtered bovines and ovine's at Addis Ababa abattoir.

Table (2): susceptibility Percentage of Salmonella sp. contamination in meat and poultry products.

Antibiotics	Susceptible parameters	Poultry products	Meat products
Ciprofloxacin	Total susceptible isolates	9	14
	% of susceptible isolates	81.8%	77.7%
Kanamycin	Total susceptible isolates	7	13
	% of susceptible isolates	63.6%	72.2%
Ampicillin	Total susceptible isolates	5	10
	% of susceptible isolates	45.4%	55.5%
Norfloxacin	Total susceptible isolates	7	7
	% of susceptible isolates	63.6%	38.3%

Specific primer considers a huge tool for microbial identification studies. For our investigation, invA gene sequence was applied as molecular marker gene through using specific complementary primer and screened for specific PCR resultant fragment with 284 bp. As shown by (photograph1, A and B), 18 and 11 Salmonella isolates from meat and poultry products amplified invA gene. After that, invA fragments were purified, sequenced and alignmented to identify Salmonella serotypes. Table (3) reflected that, Salmonella isolates from meat products were identified serotypes as Salmonella newland (12.5%), Salmonella enteritidis (77.7%) and Salmonella hessarek (9.8%). On the other hand, Salmonella serotypes which isolated from poultry products identified as Salmonella typhimurium (22.8%), Salmonella enteritidis (65.4%) and Salmonella Kentucky (11.8 %).One hundred poultry and meat products samples were screened for the presence of virulence gene (invA) by using Polymerase Chain Reaction (PCR) methodology. InvA gene was recorded and amplified in 18 and 11 Salmonella isolates form meat and poultry products respectively. More light was added to our findings by applying virulence gene (invA) as molecular marker gene by Karmi (2013). Only, 13 isolates were amplified invA gene among thirty-two samples. With accordance for using virulence gene (invA) as molecular marker for Salmonella isolates identification, Shanmugasamy et al., 2011 applied virulence gene to identify Salmonella which isolates form broiler carcasse. Employing invA gene sequence alignments via NCBI database was supported by Sharma, et al., (1995 & 1989). They recovered Salmonella serotypes (S. typhimurium, S. Typhimurium, S. Saint Paul, S. Indiana, S. Stanley, S. Derby and S. Newport) from poultry meat and S. Typhimurium from fresh buffalo meat.

Photograph (1):invAgene fragments amplification form total 50 Salmonella isolates from meat products (A) and (B) poultry products.



(A) (B)

Table (3): Salmonella serotypes isolated from meat and poultry products.

	Salmonella serotypes	Serotypes percentage
Meat products	Salmonella newland	12.5
	Salmonella enteritidis	77.7
	Salmonella hessarek	9.8
Poultry products	Salmonella typhimurium	22.8
	Salmonella enteritidis	65.4
	Salmonella Kentucky	11.8

CONCLUSION

In our investigation, 18 and 11 bacterial isolates from meat and poultry products were identified as Salmonella sp via morphological, biochemical and antimicrobial susceptibility tests. invA gene was applied as molecular marker to detect Salmonella

serotypes. Meat and poultry products Salmonella serotype identified as Salmonella newland (12.5%), Salmonella enteritidis (77.7%) and Salmonella hessarek (9.8%) and Salmonella typhimurium (22.8%),Salmonella enteritidis (65.4%) and Salmonella Kentucky (11.8 %) respectively.

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