





Benha University Faculty of Veterinary Medicine Department of Bacteriology, Immunology and Mycology

Phenotypic and genotypic characterization of some

toxigenic bacteria isolated from meat and meat products

A Thesis presented by

Nesma Mohamed Gamal Ahmed (B.V.Sc. 2011, Benha University) (M.V.Sc. 2016, Benha University)

Under The Supervision of

Prof. Dr. Ashraf Awad Abd El- Tawab

Professor and Head of Bacteriology, Immunology and Mycology, Fac. Of Vet. Med., Benha University

Dr. Fatma Ibrahim Abd – Allah El- Hofy

Assistant professor of Bacteriology, Immunology and Mycology, Fac. of Vet. Med., Benha University

Prof. Dr. Ahmed Afifi Abd El – Ghaffar Maarouf

Chief Researcher of Microbiology and Director of Animal Health Research Benha Branch

Prof. Dr. Marionette Zaghloul Nassif

Chief Researcher food Hygiene Department, Animal Health Research Institute, Benha Branch

A Thesis submitted for the Doctor of Philosophy in Veterinary Science Faculty of Vet. Med. Benha Univ. (Bacteriology, Immunology and Mycology)

(2020)

Contents

Subject	Page
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	6
2.1. Sources of contamination of meat and meat products with	
toxigenic food-borne pathogens	6
2.2. Incidence of toxigenic food-borne pathogens in meat and meat	11
products	11
2.3. Bacteriological characters of toxigenic food-borne pathogens	24
2.3.1. Bacteriological characters of <i>E. coli</i> isolates	24
2.3.1.1. Morphological and biochemical characters of <i>E. coli</i> isolates	24
2.3.1.2. Antimicrobial sensitivity and resistance of <i>E. coli</i> isolates	26
2.3.1.3. Pathogenicity and genotypic detection of virulence genes of	28
E. coli strains	20
2.3.2. Bacteriological characters of <i>S. aureus</i> isolates	30
2.3.2.1. Morphological and biochemical characters of S. aureus	30
isolates	50
2.3.2.2. Antimicrobial sensitivity and resistances of <i>S. aureus</i> isolates	33
2.3.2.3. Pathogenicity and genotypic detection of virulence genes of	26
S. aureus strains	36
2.3.3. Bacteriological characters of <i>Bacillus cereus</i> isolates	38
2.3.3.1. Morphological and biochemical characters of <i>Bacillus cereus</i>	38
isolates	30
2.3.3.2. Antimicrobial sensitivity and resistances of Bacillus cereus	40
isolates	
2.3.3.3. Pathogenicity and genotypic detection of virulence genes of	45

Bacillus cereus strains

Subject	Page
2.3.4. Bacteriological characters of Salmonella isolates	51
2.3.4.1. Morphological and biochemical characters of Salmonella isolates	51
2.3.4.2. Pathogenicity of Salmonella strains	53
3. MATERIAL AND METHODS	55
3.1. MATERIALS	55
3.1.1. Samples	55
3.1.2. Bacteriological Media	55
3.1.3. Materials used for serogrouping of <i>E. coli</i> isolates	60
3.1.4. Materials used for Anti-microbial Sensitivity test	61
3.1.5. Materials for PCR	61
3.2. Methods	68
3.2.1. Collection of the samples	68
3.2.2. Preparation of samples	68
3.2.3. Isolation of food borne pathogens	68
3.2.3.1. Isolation of <i>E. coli</i> from different samples	68
3.2.3.1.1. Identification of suspected <i>E. coli</i> isolates	69
3.2.3.2. Isolation of <i>B. cereus</i> from different samples	74
3.2.3.2.1. Identification of suspected <i>B.cereus</i> isolates	75
3.2.3.3. Isolation of <i>S. aureus</i> from different sample	76
3.2.3.3.1. Identification of the suspected S. aureus	77
3.2.3.4. Isolation of Salmonellae from different samples	79
3.2.3.4.1. Identification of the suspected Salmonella isolates	80
3.2.4. Methods of PCR technique	81
4. RESULTS	86
4.1. Isolation and identification of food borne pathogens	86
Subject	Page

4.1.1. Occurrence of positive samples for food borne pathogens isolation	86
from examined samples	
4.1.2. Percentage of foodborne pathogens isolated from examined samples	87
4.1.2.1. Percentage of <i>E. coli</i> isolates from examined samples	90
4.1.2.2. Percentage of <i>S. aureus</i> strains isolated from examined samples	92
4.1.2.3. Percentage of <i>B. cereus</i> strains isolated from examined samples	93
4.1.3. Identification of food- borne pathogens isolated from examined	94
samples	
4.1.3.1. Identification of <i>Escherichia coli</i> isolates	94
4.1.3.2. Identification of <i>Staphylococcus aureus</i> isolates	98
4.1.3.3. Identification of <i>Bacillus cereus</i> isolates	101
4.1.3.4. Identification of Salmonella isolates	104
4.2. Detection of some virulence genes in isolated <i>E. coli; S. aureus</i> and <i>B.</i>	105
cereus strains using polymerase chain reaction (PCR)	
5. DISCUSSION	114
6. CONCLUSION AND RECOMMENDATION	125
7. SUMMARY	127
8. REFERENCES	130
9. ARABIC SMARMY	-

LIST OF TABLES

Table	Title	Page
No.	The	1 age
1	Antisera used in serological identification of E. coli	62
2	Antimicrobial standardized discs, concentrations and interpretation of their effect	63
3	Oligonucleotide primers sequences	65
4	Biochemical reaction of <i>E. coli</i> isolates	72
5	The biochemical characters of the isolated members of <i>B.cereus</i> strains	76
6	The biochemical characters of the isolated members of Staphylococcus spp.	78
7	Biochemical reaction of Salmonella isolates	81
8	Preparation of uniplex PCR Master Mix	83
9	Preparation of <i>stx</i> 1, <i>stx</i> 2 duplex PCR Master Mix	83
10	Preparation of enterotoxins multiplex PCR Master Mix	84
11	Cycling conditions of the different primers during cPCR	84
12	Total number and occurrence of positive samples for pathogens isolation from studied samples	86
13	Prevalence of foodborne pathogens in examined samples	89
14	Prevalence of <i>E. coli</i> strains isolated from examined samples	91
15	Prevalence of <i>S. aureus</i> strains isolated from examined samples	92
16	Prevalence of <i>B. cereus</i> strains isolated from examined samples	93
17	Serological typing of <i>E. coli</i> strains isolated from different	96

	examined samples	
Table No.	Title	Page
18	In-Vitro anti-microbial Sensitivity test for isolated E. coli	97
19	In-Vitro anti-microbial Sensitivity test for isolated <i>S. aureus</i> strains	100
20	In-Vitro anti-microbial sensitivity test for isolated <i>B</i> . <i>cereus</i> strains	103
21	The results of PCR amplifications of different used genes of <i>E. coli</i> strains	105
22	The results of PCR amplifications of different used genes of <i>S. aureus</i> strains	108
23	The results of PCR amplifications of the different used genes of <i>B. cereus</i> isolates	110

LIST OF FIGURES

Fig.	Title	Page
No	Thic	1 age
1	Total number of positive samples for pathogen isolation from studied samples	87
2	Total Percentages of food-borne pathogens in examined samples	89
3	Incidence of food-borne pathogens in examined samples	90
4	Total number and percentage of <i>E. coli</i> isolated from examined samples	91
5	Total number and percentage of <i>S. aureus</i> isolated from examined samples	92
6	Total number and percentage of <i>B. cereus</i> isolated from examined samples	93
7	Percentage of different E. coli serotypes	96
8	In-Vitro anti-microbial Sensitivity test for isolated E. coli	98
9	In-Vitro anti-microbial Sensitivity test for isolated S. aureus	101
10	In-Vitro anti-microbial Sensitivity test for isolated <i>B</i> . <i>cereus</i> .	104
11	Agarose Gel electrophoresis of shiga toxin 1 and shiga toxin 2 genes (<i>stx</i> 1 and <i>stx</i> 2) of <i>E. coli</i>	106
12	Agarose Gel electrophoresis of edema verotoxin gene (<i>Vt2e</i>) of <i>E. coli</i>	107
13	Agarose Gel electrophoresis of Enterotoxin (<i>sea, seb, sec, sed, see</i>) genes of <i>s. aureus</i>	109
Table	Title	Page

No.		
14	AgaroseGelelectrophoresisofnon-hemolyticenterotoxin (nhe)gene	111
15	Agarose Gel electrophoresis of cytotoxic K (<i>cytK</i>) gene	112
16	Agarose Gel electrophoresis of cereulide synthetase gene (ces)	113

7. SUMMARY

Toxigenic bacterial species have been linked to major outbreaks of food poisoning, illness and death all over the world. So, the present study was conducted to throw light over these bacterial species with special reference to *E. coli*, Salmonellae; coagulase positive *S. aureus* and *B. cereus* strains in 250 random samples of fresh meat and meat products viz: Beef burger, kofta; minced meat and sausage (50 for each), were collected from different shops at Kaliobia Governorate.

The results of Food- borne pathogens isolation revealed that, 77 out of 250 samples were positive for isolation (30.8%), where 24 (31.2%) were single pure cultures and 53 (68.8%) were mixed cultures. Moreover, 129 (51.6%) isolates of foodborne pathogens were recovered from 250 samples, where *S. aureus* were the most isolated (41/16.4%) followed by *E. coli* (25 /10.0%); *B. cereus* (21/8.4%); *Enterobacter cloacae* (14/5.6%); *Citrobacter freundii* and *Kl. pneumoniae* (9/3.6% for each); *Proteus vulgaris* (7/2.8%) and Salmonellae (3/1.2%).

The results of *E. coli* isolation cleared that, 25 *E. coli* strains were isolated from minced meat samples (7/14%) followed by kofta (6/12.0%); sausage (5/10.0%); fresh meat (4/8.0%) and beef burger samples (3/6.0%). The serological examination of 25 isolated *E. coli* strains appeared that, seven isolates were typed as O55:H7 (two from each samples of kofta, minced meat and one from each samples fresh meat, beef burger, sausage) ; three O_{111} :H₄ (one from each samples of fresh meat; neat, kofta, and minced meat); five O_{125} :H₁₈ (two from minced meat; one from each samples of fresh meat; kofta and sausage) ; three O126:H7(one from each samples of kofta; minced meat and beef burger); two O128:H27 (one from each samples of fresh meat and beef burger); two O142:H2 (one from each samples of beef burger of burger burger); three O142:H2

O158:H2 (one from each samples of kofta; minced meat and sausage samples). Moreover, the results of antibiotic sensitivity tests for isolated *E. coli* showed that, they were highly resistant for methicillin followed by oxytetracycline; amoxicillin; ampicillin; streptomycin and erythromycin. But, they were highly sensitive to meropenem followed by norfloxacin; gentamycin; ciprofloxacin and florphenicol.

Meanwhile, the results of *S. aureus* isolation appeared that, 41 strains were isolated mostly from kofta samples (12 / 24.0%) followed by minced meat (9/ 18.0%), sausage, fresh meat (8/16.0% for each) and beef burger samples (4/8.0%). Moreover, the results of antibiotic sensitivity tests for isolated *S. aureus* revealed that, the isolated *S. aureus* were highly resistant for methicillin followed by ampicillin; oxytetracycline; amoxicillin; cefotaxime; streptomycin; doxycycline and erythromycin. Meanwhile, they were highly sensitive to norfloxacin followed by gentamycin; ciprofloxacin and meropenem.

Moreover, the results of *B. cereus* isolation showed that, 21 strains were isolated mostly from kofta (7/14.0%) followed by sausage (6/12.0%); minced meat (4/8.0%); beef burger (3/6.0%) and fresh meat samples (1/2.0%). The results of antibiotic sensitivity tests for isolated *B. cereus* revealed that, they were highly resistant for ampicillin; methicillin followed by oxytetracycline; amoxicillin; erythromycin and cefotaxime. Meanwhile, they were highly sensitive to gentamycin; norfloxacin followed by ciprofloxacin; meropenem and florphenicol.

The PCR results for *E. coli* strains showed that, stx^2 virulence gene was detected in one strain and vt^2e virulence gene was detected in two out of 6 studied strains, but stx^1 virulence gene was failed to be detected in all studied strains. Meanwhile, PCR results for *S. aureus* strains showed that, enterotoxin *seb* virulence gene was detected in one strain and enterotoxin *sed* virulence gene was detected in 4 out of 5 studied strains, but enterotoxins *sea*; *sec* and *see* virulence genes were failed to be detected in all studied strains. In addition, the results for *B. cereus* strains cleared that, *nhe; cyt*K and *ces* enterotoxigenic virulence genes were detected in all three studied strains.

Finally, the results proved that, the isolated *E. coli*; *S. aureus* and *B. cereus* strains are enterotoxigenic ones with multiple antibiotic resistances and they are meat-borne pathogens of public health importance.