



University of Sadat City Faculty of Veterinary Medicine (Department of Food Hygiene & Control)

Studies on Antibiotic Resistant Food Poisoning Microorganisms in Some Dairy Products.

A thesis submitted by

Eman kamal Mohamed Fathalla

(B. V. Sc. Fac. of Vet. Med., Kafer El-sheikh University., 2009) (M.V. Sc. Fac. of Vet. Med., Sadat City University., 2015)

Under supervision of

Prof. Dr. Abdel-Rahman Mahmoud El-Bagory

Professor of Milk Hygiene and Control Dean Faculty of Veterinary Medicine Monoufia University

Dr. Heba Hussein Shelaby Assistant Professor of Milk Hygiene and Control Faculty of Veterinary Medicine University of Sadat City.

Dr. Seham Nazeh Homoda

Chief Researcher of Food Hygiene, Animal Health Research Institute, Tanta, Egypt.

A thesis

Submitted to Faculty of Veterinary Medicine - University of Sadat City

For the degree of Ph.D.

(Milk Hygiene & Control)

(2019)

Contents

INTRODUCTION(1)
REVIEW OF LITRATURE(5)
MATERIAL AND METHODS (57)
RESULTS(78)
DISCUSSION(104)
CONCLUSION AND RECOMMENDATIONS(121)
SUMMARY(123)
REFRENCES(125)
ARABIC SUMMARY

List of tables

Table (1): Incidence of E. coli isolated from examined kareish cheese, ras cheese, ice
cream and milk powder samples(78)
Table (2) Serotyping of some <i>E. coli</i> strains isolated from examined Kareish cheese, ras cheese, ice cream and milk powder samples
Table (3): Antibiotic sensitivity profile of some isolated <i>E.coli</i> strains from examined samples
Table (4): Presence of some resistant genes in some antibiotic resistant <i>E.coli</i> strains isolated from examined samples
Table (5): Incidence of S. aureus isolated from examined kareish cheese, ras cheese, ice cream and milk powder samples
Table (6): Production of coagulase and TNase from isolated S. aureus strains (87)
Table (7): Antibiotic sensitivity profile of some isolated S.aureus strains from examined samples
Table (8): presence of some resistant genes in some antibiotic resistant S.aureus isolates
Table (9): Incidence of <i>Bacillus cereus</i> isolated from examined kareish, ras cheese, ice cream and milk powder samples
Table (10): Antibiotic sensitivity profile of some isolated <i>B.cereus</i> strains from examined samples
Table (11): presence of some resistant genes in some antibiotic resistant <i>B.cereus</i> isolates
Table (12): Organoleptical properties of yoghurt fortified with different ratios ofpomegranate Fruit peels and moringa oleifera leaves ethanolic extract
Table (13): Antimicrobial activity of pomegranate Fruit peels and moringa leaves extracts on E.coli count artificially inoculated in yoghurt during storage(101)

Table (14): Reduction % of *E. coli* counts artificially inoculated into yoghurt. ... (103)

List of figures

Figure (1): Incidence of *E. coli* isolated from examined kareish cheese, ras cheese, ice cream and milk powder samples......(78) Figure (2): Antibiotic sensitivity profile of some isolated *E.coli* strains from examined samples......(81)

Figure (3): agarose gel electrophoresis for amplified pcr product of *blaTem* gene in different *E.coli* isolates (DNA ladder 100- 600bp) positive control reference strain at 516 bp for *blaTem* gene which was detected at lane 1,2,3,4,5,6,7, and 8 of *E.coli* isolates...(83)

Figure (4): agarose gel electrophoresis for amplified pcr product of *sul* gene in different *E.coli* isolates (DNA ladder 100- 600bp) positive control reference strain at 433 bp for *sul* gene which was detected at lane 1,2,3,4,5,6,7, and 8 of *E.coli* isolates......(84)

Figure (5): agarose gel electrophoresis for amplified pcr product of *Dfra* gene in different *E.coli* isolates (DNA ladder 100- 600bp) positive control reference strain at 425 bp for *Dfra* gene which was detected at lane 1,2,3,4,5,6,7, and 8 of *E.coli* isolates............(85)

Figure (7): Production of coagulase and TNase from isolated S. aureus strains... (87)

Figure (9): agarose gel electrophoresis for amplified pcr product of *vanA* gene in different *S.aureus* isolates (DNA ladder 100- 1000bp) positive control reference strain at 885 bp for *vanA* gene which was detected at lane 2 of *S.aureus* isolates......(90)

Figure (10): agarose gel electrophoresis for amplified pcr product of *blaZ* gene in different *S.aureus* isolates (DNA ladder 100- 600bp) positive control reference strain at 173 bp for *blaZ* gene which was detected at lane 1,2,3,4 and 5 of *S.aureus* isolates.....(91)

Figure (12): Incidence of *Bacillus cereus* isolated from examined kareish, ras cheese, ice cream and milk powder samples......(93)

Figure (13): Antibiotic sensitivity profile of some isolated *B.cereus* strains from examined samples......(95)

Figure (15): agarose gel electrophoresis for amplified pcr product of *tetA* gene in different *B.cereus* isolates (DNA ladder 100- 600bp) positive control reference strain at 502 bp for *tetA* gene which was detected at lane 1,2,3,4 and 5 of *B.cereus* isolates......(97)

Figure (16): agarose gel electrophoresis for amplified pcr product of *ermA* gene in different *B.cereus* isolates (DNA ladder 100- 1000bp) positive control reference strain at 652 bp for *ermA* gene which was not detected at lane 1,2,3,4 and 5 of *B.cereus* isolates..(98)

Figure (17): agarose gel electrophoresis for amplified pcr product of *bla* gene in different *B.cereus* isolates (DNA ladder 100- 600bp) positive control reference strain at 680 bp for *bla* gene which was detected at lane 1,2,3,4 and 5 of *B.cereus* isolates......(99)

Figure (18): Antimicrobial activity of pomegranate Fruit peels and moringa leaves extracts on *E.coli* count artificially inoculated in yoghurt during storage......(102)

7-Summary

Two hundred samples, 50 each of Kareish cheese, Ras cheese, Ice cream and Milk powder were collected from supermarkets, dairy shops and street vendors in El- Gharbia governorate for microbiological analysis.

Microbiological examination of the examined samples revealed that *E. coli* could be detected in 32, 24, 18 and 10% of examined Kareish cheese, ras cheese, ice cream and milk powder samples, respectively.

S.aureus could be detected in 30, 22, 16 and 8% of examined Kareish cheese, ras cheese, ice cream and milk powder samples, respectively.

B.cereus could be detected in 8, 14, 10 and 20% of examined Kareish cheese, ras cheese, ice cream and milk powder samples, respectively.

Enterobacter sakazakii could not be detected in all the examined samples.

Concerning the antibiotic sensitivity test all the isolated isolates were tested against Gentamicin (120 μ g), Erythromycin (15 μ g), E-moxclav (30), Ampicillin (10 μ g), Ciprofloxacin (5 μ g), Doxycyclin (30), Cefazone (75), Sulphamethaoxazole-trimethoprim (25) and Vancomycin (30).

E.coli isolates were highly resistant to Vancomycin (30µg) and Sulphamethaoxazoletrimethoprim (25µg), while low resistant were to Ciprofloxacin (10µg) and Doxycyclin (30µg). *S.aureus* isolates recorded high resistant against Cefazone (75µg), and low resistant against Ciprofloxacin (10µg). *B.cereus* isolates were highly resistant to Cefazone (75µg), while the low resistant was to Gentamicin (120µg).

Pcr for some antibiotic resistant isolates revealed that that in *E.coli* presence of *blaTEM* (ampicillin resistant strains), *sul*1 (sulphamethoxazole resistant) and *dfr*A (trimethoprim resistant) genes in all the strains examined by PCR. *B.cereus* revealed that *TetA*(tetracyclin reistant) and *bla* (beta lactamase resistant) resistant genes were found in all the examined samples while *ermA* (erythromycin resistant) gene were not isolated. *S.aureus* showed that *mecA* (methicillin resistant) gene and *vanA* (vancomycin resistant) gene were

found in only one strain, while *blaZ* (beta lactamase resistant)gene was found in all the examined samples.

Investigation the effect of Pomegranate Fruit peels and *Moringa oleifera* leaves ethanolic extracts at concentration (1& 2% from each extract) on sensory evaluation and their inhibitory effect on EHEC (O111: H2) in yoghurt during storage, proved to all treatments and control yoghurt were acceptable and the count of *E. coli* was significally reduced during the periods of incubation.