



*Suez Canal University
Faculty of Veterinary Medicine
Department of Bacteriology, Immunology and Mycology*



"Spectroscopy as a recent technique in diagnosis of multi drug resistant E. coli causing endometritis in cattle in comparison of other traditional methods"

Thesis presented by

Ahmed Samir Mohamed El-Sherpiny

B.V.Sc., Assiute University (2008)

M.V.Sci in Veterinary Medical Science- Bacteriology, Immunology and Mycology ,

Suez Canal University (2016)

For the Degree of

Ph. D Department of Bacteriology, Immunology and Mycology

Under Supervision of

Prof. Dr.

Mohamed El Sayed Enany

*Professor of Microbiology
Bacteriology, Immunology
and Mycology Department
Faculty of Veterinary Medicine
Suez Canal University.*

Prof. Dr.

Sahar Roshdi Mohamed

*Chief Researcher
of Bacteriology
Animal Health Research Institute (AHRI)
Dokki- Giza*

A Thesis Submitted to

Faculty of Veterinary Medicine

Suez Canal University

2021

Author	Ahmed Samir Mohamed El-Sherpiny
Title	Spectroscopy as a recent technique in diagnosis of multi drug resistant <i>E. coli</i> causing endometritis in cattle in comparison of other traditional methods
Faculty	Veterinary Medicine, Suez Canal University- Ismailia - Egypt
Department	Department of Bacteriology, Immunology and Mycology
Degree	<i>Ph. D</i> in Bacteriology, Immunology and Mycology
Supervision committee	Prof. Dr. Mohamed El Sayed Enany Professor of Microbiology, Faculty of Vet. Med., Suez Canal University Prof. Dr. Sahar Roshdi Mohamed Chief Researcher of Bacteriology, Animal Health Research Institute -Dokki- Giza

Abstract

Objective: The objectives of this study were to determine the presence of some virulence and antibiotic resistant genes of *Escherichia coli* isolated from vaginal discharge samples from cattle with clinical endometritis. Also, to evaluate the performance of MALDI-TOF-MS analysis under real routine laboratory conditions.

Materials and Methods: A total of 138 vaginal discharge samples were collected from Holstein cows suffered from post-partum endometritis for bacteriological examination and serotyping identification. The antibiotic sensitivity test was carried out for *E. coli* strains by using VITEK 2 automated system. All different *E. coli* strains were chosen for genotyping characterization of some virulence and antibiotic resistant resistance genes by using PCR. Finally, MALDI-TOF-MS technique was applied for all variant *E. coli* strains to confirm its identification.

Results: A total of 93(67.4%) *E. coli* isolates were obtained by examination of 138 samples. *E. coli* serotypes which obtained were O126, O55, O26, O86a, O63, O119, O111, O15, O114 and O142. The results of antibiotic sensitivity test revealed that, Ampicillin / Sulbactam, Piperacillin / Tazobactam, Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Ciprofloxacin, Levofloxacin and Nitrofurantoin were the most effective antibiotics against all *E. coli* strains (100%). On the other hand, *E. coli* isolates resisted the action of Ampicillin, Amoxicillin / clavulanic acid, Tetracycline and Streptomycin (100%). The genotyping characterization of *E. coli* resistance and virulence genes by using PCR proved that, all strains harbored *phoA*, *fimH*, *blaTEM* & *aadA1*. And only O55, O86a, O111, O114 & O142 harbored *eaeA*. On the other hand, *kpsMII* & *ibeA* genes didn't detected within any strains. MALDI-TOF-MS technique confirmed the isolates of *E. coli* with interpretation high confidence and performed the dendrogram for all *E. coli* strains according to the similarities of peaks which observed within each *E. coli* spectrum.

Keywords: endometritis - *E. coli* - VITEK2- MALDI-TOF-MS – virulence - dendrogram

List of Contents

Content No.	Title	Page No.
1	Introduction	1
2	Review of Literature	6
2.1	Normal post-partum events in cattle	6
2.2	Uterine infections in cattle	7
2.3	Endometritis in cattle	8
2.4	Prevalence of pathogenic <i>E. coli</i> in cattle suffering from clinical endometritis	12
2.5	Characterization and classification of <i>E. coli</i>	16
2.6	Serotyping of <i>E. coli</i> strains	18
2.7	VITEK 2 automated system application in bacteriological field	20
	Antibiotic sensitivity tests for <i>E. coli</i> strains	20
2.8	Molecular characterization of <i>E. coli</i> strains by using Polymerase Chain Reaction (PCR)	23
2.8.1	Detection of <i>E. coli</i> virulent genes	23
2.8.2	Detection of antibiotic resistant genes	30
2.9	Matrix –Assisted Laser Desorption/Ionization Time of Flight mass spectrometry (MALDI-TOF-MS) for identification of bacterial species	32
3	Material and Methods	42
3.1	Materials	42
3.1.1	Samples	42
3.1.2	Material used for phenotypic characterization of <i>E. coli</i>	43
3.1.2.1	Media used for <i>E. coli</i> isolation and identification	43
3.1.2.2	Media used for preservation and detection of the isolate's motility	46
3.1.2.3	Media used for biochemical tests according to	47
3.1.3	Gram's stain	50
3.1.4	Reagents and chemicals for biochemical tests	50
3.1.5	<i>E. coli</i> Antisera	52

3.1.6	Material used for detection of antibiotic sensitivity test for isolated <i>E. coli</i> by using VITEK2 automated microbiology system	53
3.1.7	Material used for molecular characterization of <i>E.coli</i> by using cPCR	55
3.1.7.1	Material used for extraction of DNA	55
3.1.7.2	Equipment and apparatuses used for extraction of nucleic acids	55
3.1.7.3	PCR Master Mix used for conventional PCR	55
3.1.7.4	Oligonucleotide primers used in cPCR	55
3.1.7.5	DNA Molecular weight marker	56
3.1.7.6	Material used for agarose gel electrophoresis	57
3.1.7.7	Equipment and apparatuses used in cPCR	58
3.1.8	Materials used for identification of <i>E. coli</i> by using Matrix-Assisted Laser Desorption / Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS)	59
3.2	Methods	60
3.2.1	Isolation and identification of <i>E. coli</i>	60
3.2.1.1	Colonial morphology	60
3.2.1.2	Microscopical examination	60
3.2.1.3	Motility test	60
3.2.1.4	Biochemical tests	60
3.2.2	Serotyping of <i>Escherichia coli</i> isolates	62
3.2.3	Detection of antibiotic sensitivity tests for isolated <i>E. coli</i> serotypes by using VITEK2 automated microbiology system	63
3.2.3.1	Suspension preparation	63
3.2.3.2	Inoculation	64
3.2.3.3	Card sealing and incubation	64
3.2.3.4	Optical system	65
3.2.3.5	Test reactions	65
3.2.3.6	Database development	65
3.2.4	Molecular characterization of <i>E. coli</i> by using conventional polymerase chain reaction (cPCR)	65
3.2.4.1	Extraction of DNA	65

3.2.4.2	Preparation of PCR Master Mix	66
3.2.4.3	Cycling conditions of the primers during cPCR	67
3.2.4.4	DNA Molecular weight marker	68
3.2.4.5	Agarose gel electrophoreses	68
3.2.5	Identification of <i>E. coli</i> by using Matrix-Assisted Laser Desorption / Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS)	68
4	Results	71
4.1	The incidence of <i>E. coli</i> in lochia of cattle suffering from clinical endometritis	71
4.2	Phenotypic characterization of <i>E. coli</i>	72
4.2.1	Detection of the morphological properties of <i>E. coli</i> in different media	72
4.2.2	Detection of the microscopic properties of <i>E. coli</i> by gram stain	73
4.2.3	Motility test	73
4.2.4	Biochemical tests	73
4.3	Serotyping of <i>E. coli</i> isolates	75
4.4	Antibiotic sensitivity testing of <i>E. coli</i> strains	76
4.5	Genotyping characterization of <i>E. coli</i> by using polymerase chain reaction (PCR)	79
4.5.1	Conventional polymerase chain reaction (c PCR) for detection of Alkaline phosphatase gene (<i>phoA</i>)	79
4.5.2	Conventional polymerase chain reaction (c PCR) for detection of virulence genes (Fimbrin like protein H gene (<i>fimH</i>), Invasion and biofilm formation gene (<i>ibeA</i>), Capsular gene (<i>kpsMTII</i>) and Intamin gene (<i>eaeA</i>)) of <i>E.coli</i> strains	80
4.5.3	Conventional polymerase chain reaction (c PCR) for detection of Antibiotics resistant genes (nonspecific TEM B – lactamase gene (<i>blaTEM</i>), and streptomycin resistant gene (<i>aadA1</i>)) of <i>E. coli</i> strains	82

4.6	Identification of <i>E. coli</i> by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	85
5	Discussion	89
6	Conclusion	105
7	Recommendation	106
8	Summary	108
9	References	110
10	الملخص العربي	1

List of Tables

Tables No.	Table Title	Page No.
1	Total number of collected samples and their sources.	42
2	<i>Escherichia coli</i> antisera.	52
3	Oligonucleotide primers sequences.	56
4	Suspension Turbidities Used for Card Inoculation.	63
5	PCR Master Mix component.	67
6	Cycling conditions of the primers during cPCR.	67
7	The incidence of <i>E. coli</i> isolated from cattle suffering from clinical endometritis.	71
8	The results of different biochemical testes on <i>E. coli</i> isolates.	73
9	Prevalence of <i>E. coli</i> serotypes.	75
10	The results obtained by using VITEK2 system for detection antibiotic sensitivity of <i>E. coli</i> strains.	76
11	The results obtained by using VITEK2 system for detection antibiotic sensitivity of <i>E. coli</i> strains.	77
12	The percentage of sensitive <i>E. coli</i> strains for different antimicrobial agent.	78
13	Incidence of <i>phoA</i> gene, virulence genes (<i>fimH</i> , <i>eaeA</i> , <i>ibeA</i> & <i>kpsMTII</i>) as well as antibiotics resistant genes (<i>blaTEM</i> & <i>aadA1</i>) among <i>E. coli</i> strains by c PCR.	84
14	The results of <i>E. coli</i> isolats confirmation by MALDI -TOF- MS.	85
15	The interpretation of scores values.	85

List of Figures

Figure No.	Figure Title	Page No.
1	The appearance of vaginal discharge from cattle suffering from endometritis.	43
2	The appearance of different type of abnormal vaginal discharge.	43
3	VITEK® Compact Instrument	54
4	VITEK® 2 AST-GN38 cards within Cassette	54
5	Ultraflex LT mass spectrometer (Bruker Daltonics)	59
6	Target plate (Bruker Daltonics)	59
7	Schematic representation of the time-of-flight mechanism; the mixture of analyte and matrix are targeted by laser beam and analyte ions are accelerated and led towards the ion detector at the end of flight zone	70
8	Linear mode MALDI-TOF-MS; heavier ions arrive to the destination later than lighter ions.	70
9	Number of positive <i>E. coli</i> isolates obtained from cattle with clinical endometritis	72
10	Percentage of positive <i>E. coli</i> isolates from cows suffering from clinical endometritis	72
11	<i>E. coli</i> in MacConkey agar appeared as 1-2 mm diameter pink colonies	74
12	<i>E. coli</i> in EMB agar shows characteristic greenish metallic colonies	74
13	(Motility test) <i>E. coli</i> on semisolid nutrient agar appeared as +ve motility test (right section)	74
14	Indole test showed positive result which confirmed by appearance of pink ring	74
15	Negative result detected by culturing <i>E. coli</i> on Simmon's citrate agar (no color change)	74
16	<i>E. coli</i> on TSI agar show acid butt and slant with CO ₂ formation and without H ₂ S production	74
17	Percentage of <i>E. coli</i> serotypes	75
18	Percentage of sensitive <i>E. coli</i> strains to different antibiotics	78

19	Agarose gel electrophoresis of amplified <i>phoA</i> gene PCR product with specific amplicon size (720bp)	79
20	Agarose gel electrophoresis of amplified <i>fimH</i> gene PCR product with specific amplicon size (508bp)	80
21	Agarose gel electrophoresis of amplified <i>eaeA</i> gene PCR product with specific amplicon size (248bp)	81
22	Agarose gel electrophoresis of amplified <i>ibeA</i> gene PCR product with specific amplicon size (342bp)	81
23	Agarose gel electrophoresis of amplified <i>kpsMTII</i> gene PCR product with specific amplicon size (280bp)	82
24	Agarose gel electrophoresis of amplified <i>blaTEM</i> gene PCR product with specific amplicon size (516bp)	83
25	Agarose gel electrophoresis of amplified <i>aadA1</i> gene PCR product with specific amplicon size (484bp)	83
26	Percentage of Incidence of <i>phoA</i> gene, virulence genes (<i>fimH</i> , <i>eaeA</i> , <i>ibeA</i> & <i>kpsMTII</i>) as well as antibiotics resistant genes (<i>blaTEM</i> & <i>aadA1</i>) among <i>E.coli</i> strains by c PCR	84
27	The Spectra obtained by MALDI ToF ms analysis specific for <i>E. coli</i> serotype O119	86
28	Different <i>E. coli</i> serotypes mass spectra	87
29	Dendrogram analysis for all <i>E. coli</i> serotypes subjected to identification by using MALDI-TOF-MS	88