UNIVERSITI PUTRA MALAYSIA

OCCURRENCE, ANTIBIOTIC RESISTANCE AND GENETIC DIVERSITY OF ARCOBACTER ISOLATES FROM CATTLE AND GOATS

SAYED ATTA HUSSAIN SHAH

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By

SAYED ATTA HUSSAIN SHAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

June 2012
DEDICATIONS

I would like to dedicate this work to

My Family

Who are my inspiration, my heart and my strength

Without their spur and impulse

I would not have the goals I have to strive for and be the best to
reach my dreams!
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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June 2012

Chairman: Prof. Saleha Abdul Aziz, PhD
Faculty: Veterinary Medicine

Arcobacter is considered as one of the emerging food and water borne zoonotic pathogens. It has been isolated from various species of animals, animal origin food products, vegetables, and water. Most of the studies on detection and characterization of Arcobacter have been carried out in developed countries whereas such data is lacking in developing countries like Malaysia. With this scenario in view, this study was carried out to isolate Arcobacter spp. from cattle, goats, milk, beef and water, to determine its antimicrobial resistance patterns and to evaluate the genetic diversity of the isolates.

A total of six dairy cattle farms in Rawang (A), UPM (B), Serdang (C), Kuala Kubu Baru (D), and Sepang (E) in Selangor and in Tampin (F) in Negeri Sembilan were visited for collection of cattle rectal swabs (adults, n=120; young=120), water (n=18) and environmental surfaces (n=30) samples. The occurrence of Arcobacter in adult cattle on farm A, B, C, D, E and F was recorded as 10% (2/20), 5% (1/20), 0% (0/20),
0% (0/20), 10% (2/20) and 15% (3/20), respectively whereas for young cattle the occurrences were, 5% (1/20), 0% (0/20), 10% (2/20), 10% (2/20), 0% (0/20) and 5% (1/20), respectively. The overall prevalence of *Arcobacter* in adult cattle was 6.7% (8/120) and in the young was 5% (6/120) which was non-significant (p=0.584). *Arcobacter* was not detected in floor samples collected from farm A, however 40% of samples from farm B, C, D and F each, and 20% from farm E were detected positive. *Arcobacter* was not detected from treated water from all farms except farm F (66.7%). In total, 8 of 30 (26.66%) floor samples and 2 of 18 (11.11%) treated water samples examined were positive for *Arcobacter*. Overall, *Arcobacter butzleri* was the most frequently isolated species 9/24 (37.5%) followed by *Arcobacter skirrowii* 1/24 (4.1%).

A total of 140 samples including goat rectal swabs (n=100), water (n=15) and floor swabs (n=25) were collected from five goat farms in Rawang, Sepang, Kuala Kubu Baru, Cyberjaya in Selangor and Nilai in Negeri Sembilan and found negative for *Arcobacter* spp.

A total of 148 beef samples, local (n=85) and imported (n=63) and 180 milk samples, cattle (n=86) and goats (n= 94) were collected. Overall, 26.35% (39/148) beef samples were found positive for *Arcobacter*. Imported beef was more contaminated (39.68%) than local (16.47%) beef. *Arcobacter butzleri* was the most frequent species isolated from imported (52%) and local (35.71%) beef. *Arcobacter* was also detected from cattle milk (5.43%) with *A. butzleri* as the dominant species (60%) followed by *A. cryaerophilus* (40%). None of the goat milk samples was found positive for *Arcobacter*. 
Using minimum Inhibitory Concentration Evaluator (M.I.C.E) and disc diffusion methods, the A. butzleri isolates from cattle (n=5), beef (n=15), milk (n=3), water (1) and floor (n=2) were tested against six antibiotics namely ampicillin, ciprofloxacin, erythromycin, tetracycline, cefotaxime and gentamicin. None of the A. butzleri isolates was found resistant to all six antibiotics tested. A wide range of A. butzleri isolates were found resistant to antibiotics using both techniques: 74.07-81.48% for ampicillin, 22.22-25.92% for ciprofloxacin, 37.03-70.37% for erythromycin, 7.4-11.11% for tetracycline, 55.55-70.37% for cefotaxime and 18.51-22.22% for gentamicin, respectively. Of the resistant isolates, 3.70% and 11.11% isolates were found MDR using M.I.C.E and disc diffusion methods, respectively. The lowest minimum inhibitory concentrations (MICs), MIC<sub>50</sub>/MIC<sub>90</sub> values were obtained for tetracycline (0.03/4 µg/mL) however highest for ampicillin (32/128 µg/mL). The results of the comparison of two agar diffusion based antimicrobial susceptibility methods, M.I.C.E and disc diffusion, revealed high relationship (R ≥0.9) for five of six antibiotics tested whereas the relationship of two methods was moderate (R=0.463) for erythromycin.

Arcobacter butzleri isolates from cattle (n=5), beef (n=15), milk (n=3), water (1) and floor (n=2) were characterized by pulsed field gel electrophoresis (PFGE) using EagI restriction endonuclease and multilocus sequence typing (MLST) and high genetic diversity was observed. PFGE analysis of isolates from various sources revealed six major clusters with 16 genotypes having 50-71% similarity. Similarly high genetic diversity was seen when multilocus sequence typing was performed for two representatives of each source (cattle, beef and milk) and the results revealed that 16 of 42 (38.09%) alleles were novel alleles, which brought six new sequence types.
(STs). The overall dn/ds ratio was less than one (<1). When these new STs were compared with available electronic database, it showed that ST-366 (beef) was closely related (71.42%) with ST-116 which was isolated from pork in Thailand.

It is concluded that the occurrence of *Arcobacter* spp. in animals and animal origin food products such as beef and milk, and treated water is of public health significance. Good management and hygienic practices are key factors to control the occurrence of *Arcobacter* at farm and market levels, respectively. In addition, antimicrobial resistance in *Arcobacter* spp. against commonly used antibiotics in human and veterinary therapy may increase risk of treatment failure. Tetracycline may be used as a drug of choice against *Arcobacter* infection. High genetic diversity in *A. butzleri* genome suggested that the animals and their by products were colonized by multiple *Arcobacter* parent genotype.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEHADIRAN, KERINTANGAN ANTIBIOTIK DAN KEPELBAGAIAN GENETIK ARCObACTER YANG DIASINGKAN DARIPADA LEMBU DAN KAMBING

Oleh

SAYED ATTA HUSSAIN SHAH

Jun 2012

Pengerusi:  Prof. Saleha Abdul Aziz, PhD
Fakulti:  Perubatan Veterinar

Terdapat pandangan yang meningkat mengenai Arcobacter sebagai salah satu patogen zoonotik yang baru muncul pada makanan dan air. Ia telah diasingkan daripada pelbagai spesis haiwan, produk makanan asal haiwan, sayur-sayuran, dan air. Kebanyakan kajian ke atas pengesanan dan pencirian keatas Arcobacter telah dijalankan di negara-negara maju manakala data ini kurang didapati di negara-negara membangun seperti Malaysia. Dengan pandangan senario sebegini, kajian tersebut dijalankan untuk mengasingkan Arcobacter spp. daripada lembu, kambing, susu lembu, dan air, menentukan corak rintangan antibiotik dan untuk menilai kepelbagaian genetik isolat.

Sejumlah enam ladang lembu tenusu di Rawang (A), UPM (B), Serdang (C), Kuala Kubu Baru (D), dan Sepang (E) di Selangor dan di Tampin (F) di Negeri Sembilan telah dilawati untuk mengambil sampel rektun lembu (dewasa, n=120; muda=120), air (n=18) dan permukaan lantai kandang (n=30). Kehadiran Arcobacter dalam lembu
dewasa di ladang A, B, C, D, E dan F masing-masing adalah 10% (2/20), 5% (1/20), 0% (0/20), 0% (0/20), 10% (2/20) dan 15% (3/20) manakala bagi lembu muda kehadiran masing-masing adalah 5% (1/20), 0% (0/20), 10% (2/20), 10% (2/20), 0% (0/20) dan 5% (1/20). Secara keseluruhan, kehadiran Arcobacter lembu dewasa adalah 6.7% (8/120) dan lembu muda adalah 5% (6/120) yang tak signifikan (p=0.584). Arcobacter tidak dikesan pada sampel lantai yang ambil daripada ladang A manakala 40% daripada setiap ladang B, C, D dan F, dan 20% dari ladang E. Arcobacter tidak dikesan pada air yang dirawat di mana-mana ladang kecuali ladang F (66.7%). Secara keseluruhan, 8 daripada 30 (26.66%) sampel lantai dan 2 daripada 18 (11.11%) sampel air yang diperiksa didapati positif untuk Arcobacter. Arcobacter butzleri adalah spesis yang paling kerap diasingkan iaitu 9 daripada 24 (37.5%) dan diikuti oleh Arcobacter skirrowii, 1 daripada 24 (4.1%). Keseluruhan, Arcobacter butzleri adalah spesies yang paling kerap diasing 9/24 (37.5%) diikuti oleh Arcobacter skirrowii 1/24 (4.1%).

Sejumlah 140 sampel swab rektum kambing (n = 100), air (n = 15) dan swab lantai (n=25) diambil daripada lima ladang kambing iaitu di Rawang, Sepang, Kuala Kubu Baru, Cyberjaya dan. Tiada sampel daripada ladang kambing tersebut didapati positif untuk Arcobacter.

Sejumlah 148 sampel daging lembu tempatan (n=85) dan import (n=63) dan 180 sampel susu, lembu (n=86) dan kambing (n=94) telah diambil. Secara keseluruhan, 26.35% (39/148) sampel daging lembu didapati positif untuk Arcobacter. Daging lembu import adalah lebih tercemar (39.68%) daripada daging lembu tempatan (16.47%). Arcobacter butzleri adalah spesis yang paling kerap diasingkan daripada
daging lembu import (52%) berbanding dengan tempatan (35.71%). Hanya satu sampel daging lembu tempatan (10%) didapati positif bagi *A. skirrowii*.* Arcobacter* dikesan pada susu lembu (5.43%) dengan *A. butzleri* sebagai spesis dominan (60%) diikuti oleh *A. cryaerophilus* (40%). Tiada sampel susu kambing yang didapati positif bagi *Arcobacter*.

Minimum Inhibitory Concentration Evaluator (M.I.C.E) dan kaedah *disc diffusion* digunakan keatas *Arcobacter* bagi menentukan kerintangan daripada lembu (n = 5), daging lembu (n = 15), susu (n = 3), air (1) dan lantai (n = 2) terhadap enam antibiotik iaitu ampicillin, ciprofloxacin, erythromycin, tetracycline, cefotaxime dan gentamicin. Didapati tiada *A. butzleri* yang diasingkan tahan terhadap semua enam antibiotik yang diuji. Julat *A. butzleri* ditemui tahan kepada antibiotik menggunakan kedua-dua kaedah, adalah masing-masing: 47.07-81.48% untuk ampicillin, 22.22-25.92% untuk ciprofloxacin, 37.03-70.37% untuk erythromycin, 7.4-11.11% untuk tetracycline, 55.55-70.37% bagi cefotaxime dan 18.51-22.22% untuk gentamicin. Hasil M.I.C.E menunjukkan bahawa sejumlah 22 daripada 27 (81.48%) *A. butzleri* yang tahan terhadap satu sampai tiga antibiotik dan 1 daripada 27 (3.70%) tahan terhadap empat atau lima antibiotik yang diuji. Nilai MIC<sub>50</sub> dan MIC<sub>90</sub> yang terendah direkodkan untuk tetracycline (0.03/4 µg/mL) manakala ampicillin (32/128 µg/mL) menghasilkan nilai tertinggi. Hasil daripada perbandingan kedua kaedah didapati perbezaan yang hubungan yang tinggi (R ≥ 0.9) keatas lima daripada enam antibiotik yang diuji, manakala sederhana (R= 0.463) untuk erythromycin.

Sebanyak 27 isolat *A. butzleri*, lembu (n = 5), daging lembu (n = 15), susu (n = 3), air (1) dan lantai (n = 2) dimenciri dengan kaedah *Pulsed Field Gel Electrophoresis*
(PFGE) menggunakan endonuclease sekatan EagI dan multilocus sequence technique (MLST), dan kepelbagaian genetik yang tinggi telah diperhatikan. Analisis PFGE keatas pencilan daripada pelbagai sumber mendedahkan enam kelompok utama dengan 16 genotip mempunyai persamaan 50-71%. Begitu juga kepelbagaian genetik yang tinggi pada A. butzleri dapat diperhatikan apabila dua contoh dari setiap sumber (lembu, daging lembu dan susu) telah menggunakan multilocus sequence typing (MLST). Hasil menunjukkan bahawa 16 daripada 42 (38,09%) alel merupakan alel yang baru, yang membawa kepada enam tip sekuens (STs) baru. Nisbah Dn/ds nisbah keseluruhan adalah kurang daripada satu. Perbandingan STs baru dengan pangkalan data elektronik yang tersedia ada menunjukkan bahawa ST-366 (daging lembu) berkait rapat dengan ST-116 (57.14%) yang telah diasingkan daripada daging babi dari Thailand.

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SAYED ATTA HUSSAIN SHAH
I certify that a Thessis Examination Committee has met on 12 June, 2012 to conduct the final examination of Sayed Atta Hussain Shah on his thesis entitled “Occurrence, antibiotic resistance and genetic diversity of Arcobacter isolates from cattle and goats” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Dr. Rasedee Mat bin Abdullah, Ph.D
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Dr. Abdul Rahim bin Abdul Mutalib, Ph.D
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Dr. Siti Khairani Binti Bejo, Ph.D
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Dr. Hussni Omar Mohammed, Ph.D
Professor
College of Veterinary Medicine
Cornell University, United States
(External Examiner)

____________________________________________________________

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Saleha Abdul Aziz, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Zunita Zakaria, PhD**  
Assoc. Professor  
Faculty Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Murugaiyah Marimuthu, PhD**  
Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, Ph.D**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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SAYED ATTA HUSSAIN SHAH

Date: 12 June 2012
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