UNIVERSITI PUTRA MALAYSIA

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

SAYED ATTA HUSSAIN SHAH

FPV 2012 21

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DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2012

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By

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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!

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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

By

SAYED ATTA HUSSAIN SHAH

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),

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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*.

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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_{50}/MIC_{90} 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 ($R \geq 0.9$) 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

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(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.

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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

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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

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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 dengan 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, 7.4-11.11.11% 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_{50} dan MIC_{90} 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 perbezan yang hubungan yang tinggi ($R \geq 0.9$) 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*

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(PFGE) menggunakan endonuclease sekatan *Eag1* 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 dasing babi dari Thailand.

Dapatlah disimpulkan bahawa kehadiran spesis *Arcobacter* pada haiwan dan makanan yang berasal dari haiwan seperti daging lembu dan susu dan air mempunyai kepentingan kesihatan awam. Pengurusan yang baik dan amalan kebersihan adalah faktor utama untuk mengawal berlakunya *Arcobacter* di peringkat ladang dan pasaran. Ditambah pula ketahanan *Arcobacter* spp. terhadap antibiotik yang biasa digunakan dalam terapi manusia dan veterinar boleh meningkatkan risiko kegagalan rawatan. Tetracycline boleh digunakan sebagai antibiotik pilihan terhadap jangkitan *Arcobacter*. Kepelbagaian genetik yang tinggi dalam *A. butzleri* mencadangkan bahawa haiwan dan produk mereka telah dijajah oleh genotip induk *Arcobacter* yang pelbagai.

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ACKNOWLEDGEMENTS

First and foremost, I would expresses humblest and sincerest words of gratitude to Almighty Allah, Who bestowed the potential and ability upon me to bring the trance into reality. Secondly, I am truly indebted and thankful to my parents who instilled enthusiasm in me and sacrificed their sentiments to refine my life.

I owe sincere and earnest thankfulness to Prof. Dr. Saleha Abdul Aziz, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia for being a committed supervisor. The dream of mine, to get doctorate degree, would not have come true if she has not guided, constructively criticized and encouraged during the entire period of my research.

I am indebted to supervisory committee members, Assoc. Prof. Dr. Zunita Zakaria, Department of Veterinary Pathology and Microbiology, and Dr. Murugaiyah Marimuthu, Department of Clinical studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia who kept on encouraging me and provided valuable suggestions throughout studies and research work. I would like to express gratitude to Assoc. Prof. Dr. Cheah Yoke Kqueen, Associate Professor, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, who remained my mentor and provided enough support to accomplish my goal.

I would like to show my gratitude to the staff of Veterinary Public Health Lab and Bacteriology Lab for their cooperation and unreserved assistances throughout my laboratory work. I would like to extend thankfulness to my lab mates for their assistance and friendly cooperation during my lab work.

Thanks goes to the Worldbank for providing partial support under Robert S. McNamara Fellowship Program to the project. I am also grateful to the Sindh Agriculture University Tandojam for providing oppertunity to accomplish doctorate degree.

Last but not the least, I would like to express gratitude to worthy gift “friends” of Almighty Allah, for uplifting my morale during the entire lonely journey of Ph.D without which I would not have achieved one of the goals in my life.

SAYED ATTA HUSSAIN SHAH

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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.

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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:

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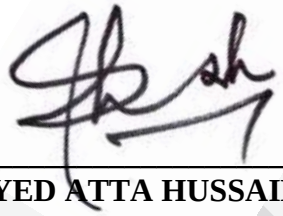
DECLARATION

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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