



UNIVERSITI PUTRA MALAYSIA

**PROFILING OF VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE
GENES AMONG PHYLOGENETIC GROUPS OF EXTENDED-SPECTRUM-
BETA-LACTAMASE PRODUCING *Escherichia coli***

KHADEGA YAHYA ABDULLAH AL-HETAR

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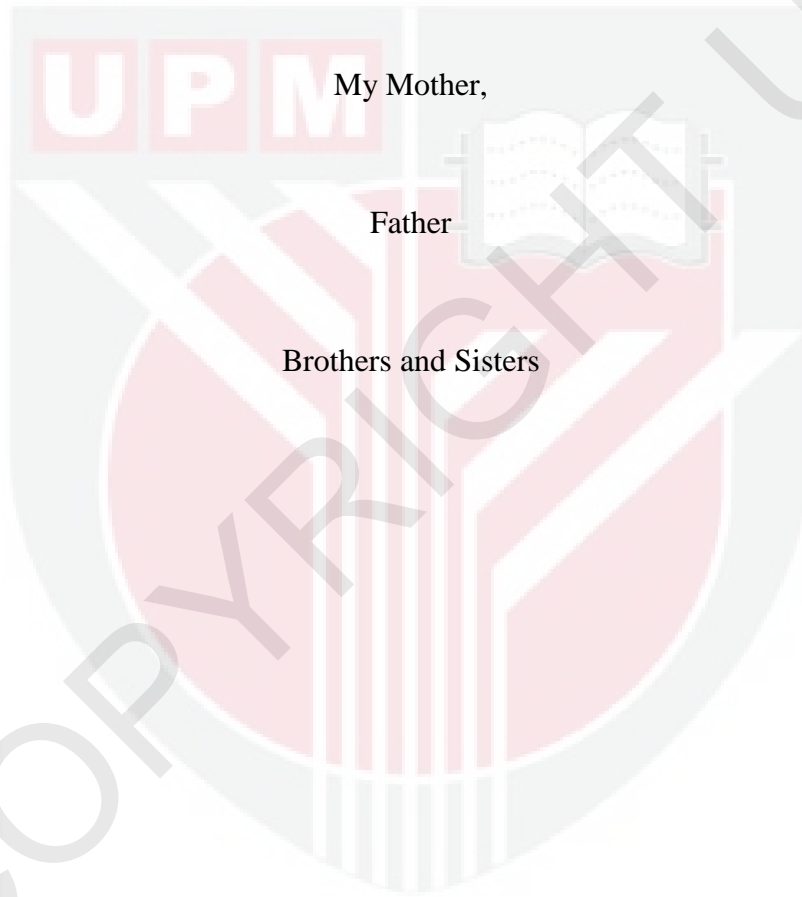
By

KHADEGA YAHYA ABDULLAH AL-HETAR

**Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

DEDICATED

To



Abstract of thesis presented to the senate of University Putra Malaysia in fulfilment of the requirements of the degree of Master of Science

PROFILING OF VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE GENES AMONG PHYLOGENETIC GROUPS OF EXTENDED-SPECTRUM-BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI*

By

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May 2011

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Extra-intestinal infection caused by *Escherichia coli* arose in recent years throughout the world. Dissemination of antibiotic resistant strains especially resistant to beta-lactams make the *E. coli* infections difficult to be treated. Presence of commensal *E. coli* that are resistant to beta-lactams among extraintestinal infections plays an important role in disseminating antibiotic resistance among different microorganisms.

Phylogenetically *E. coli* are classified into four main phylogenetic groups A, B1, B2 and D by the presence of *chuA*, *yjaA* and *TsPE4C2* genes. The virulent extra-intestinal strains mainly belong to group B2, and to a lesser extent to group D and harbor many types of virulence genes, whereas most commensal strains belong to group A and B1 with low virulence genes. The ESBLs are known to be produced more in low virulent strains than in high virulent strains. It is

important to determine the factors that make commensal *E. coli* resistant to drug and also cause infections. Therefore the goal of the current study is to investigate the association between antibiotic resistance and virulence factors among different phylogenetic groups which play a vital role in the management of infections.

In the current study 95 clinical isolates of Extended spectrum beta-lactamase (ESBL)-*E. coli* collected from various infections from Selayang Hospital in Malaysia were investigated. The isolates were phylogenetically classified, followed by ESBL typing and important virulent determinants such as adhesions and toxins determination. Among the 95 isolates 42 (44%), 26 (27%), 14 (14.7%) and 13 (13.7%) belonged to groups D, A, B2 and B1. Based on the antibiotic susceptibility test most isolates showed resistance to antibiotics as following; cefotaxime (n=78; 82.11%), ampicillin/sulbactam (78; 82.11%), sulfamethaxazole/trimethoprim (77; 81.1%), azetronem (70; 73.7%), cefepime (53; 55.79%), and ampicillin (n=95; 100%).

Investigation on the genes coding for the beta-lactamase enzymes among the *E. coli* isolates showed that majority (58.9%) belonged to the *CTX-M-1*-type with *CTX-M-15* as the predominant sub type. *CTX-M-9* cluster was significantly higher among pathogenic group than commensal group ($P < 0.05$), while *CTX-M-1* cluster was found more among commensal (61.5%) than pathogenic (57.7%) group. However, no significant association between the groups was observed ($P > 0.05$). *TEM* was detected in 8 (8.4%) isolates while *SHV* was rare; twelve (12.6%) isolates did not carry any resistance genes.

Among the six virulence genes (*iutA*, *hlyA*, *sfa/foc*, *afa/dra*, *kpsMII* and *pap*) investigated in the ESBL-*E. coli* isolates, *iutA* was found to be more prevalent (n=56, 59%) followed by *kpsMII* (n=33, 34.7%). Genes coding for *afa/foc* was not detected in any isolate while *sfa/foc* and *hlyA* were rarely seen. Among the total isolates (n=95) tested 27 (28%) did not carry any of virulence factors. Analyzing the association between pathogenic and commensal groups for *iutA* did not show any significant association for pathogenic and commensal ($P>0.05$) while the *kpsMII* was highly significant ($P<0.05$) for pathogenic group.

In conclusion isolates of *CTX-M* types producing virulent *E. coli* strains among commensal groups isolated from extra-intestinal infections warrants the urgent need for the control of such pathogen as it not only increases the infection rate but also has the potential to spread antibiotic resistance and virulence to other nosocomial pathogens.

**PEMPROFILAN FAKTOR VIRULEN DENGAN GEN RINTANG
ANTIBIOTIK DI KALANGAN KUMPULAN PHYLOGENETIK
ESCHERICHIA COLI YANG MENGHASILKAN BETA-LAKTAMASE-
SPEKTRUM-TERPERLUAS**

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E. coli yang menyebabkan jangkitan luar usus timbul beberapa tahun kebelakangan ini di serata dunia. Penyebaran strain rintang antibiotik terutamanya rintang terhadap beta-laktam menjadikan jangkitan *E. coli* susah untuk dirawat. Kehadiran *E. coli* komensal yang rintang terhadap beta-laktam dalam jangkitan luar usus memainkan peranan penting dalam menyebarkan rintangan terhadap antibiotik di kalangan mikroorganisma berlainan.

Secara phylogenetiknya, *E. coli* dikelaskan ke dalam empat kumpulan phylogenetik utama iaitu A, B1, B2, dan D berdasarkan kehadiran gen-gen *chuA*, *yjaA*, dan *TsPE4C2*. Strain luar usus yang virulen kebanyakannya adalah daripada kumpulan B2, dan adakalanya daripada kumpulan D, dan membawa bersama berjenis-jenis gen virulen sementara kebanyakan strain komensal adalah daripada kumpulan A dan B1 dan mempunyai gen-gen virulen yang rendah. Adalah penting untuk menentukan faktor-faktor yang menjadikan *E. coli* komensal rintang terhadap ubat dan menyebabkan jangkitan. Oleh itu, matlamat kajian ini adalah untuk menyiasat hubungkait di

antara rintangan terhadap antibiotik dengan factor-faktor virulen di kalangan kumpulan phylogenetik berlainan yang memainkan peranan penting dalam pengurusan jangkitan.

Dalam penyelidikan ini, 95 isolat klinikal *E. coli*-ESBL yang diambil daripada pelbagai jangkitan dari Hospital Selayang telah dikaji. Isolat-isolat ini telah dikelaskan secara phylogenetik, dan jenis ESBL serta penentu virulan penting yang merangkumi pelekatan dan toksin telah ditentukan. Daripada 95 isolat itu, 42 (44%), 26 (27%), 14 (14.7%) dan 13 (13.7%) adalah daripada kumpulan D, A, B2 dan B1 masing-masing. Berdasarkan ujian rentan antibiotik, kebanyakan daripada isolat menunjukkan rintangan terhadap antibiotik-antibiotik berikut: cefotaxime 78 isolat (82.11%), ampicillin/sulbactam 78 isolat (82.11%), sulfamethazole/trimethoprim 77 isolat (81.1%), azetronem 70 isolat (73.7%), cefepem 53 isolat (55.79%), dan ampicillin 95 isolat (100%).

Siasatan ke atas gen-gen yang mengkod untuk enzim beta-laktamase di kalangan isolat *E. coli* menunjukkan kebanyakan daripadanya (58.9%) adalah daripada *CTX-M-1* dengan *CTX-M-15* sebagai prajenis yang dominan. Gugusan *CTX-M-9* menunjukkan kehadiran yang ketara di kalangan kumpulan patogenik berbanding dengan kumpulan komensal ($P=0.005$), sementara gugusan *CTX-M-1* lebih ditemui di kalangan komensal (61.5%) daripada kumpulan patogenik (57.7%). Walaubagaimanapun, tiada perbezaan ketara diperhatikan di antara kedua-dua kumpulan ($P>0.05$). *TEM* telah dikesan dalam 8 (8.4%) isolat sementara SHV jarang ditemui dan 12 (12.6%) isolat tidak mempunyai apa-apa gen rintangan.

Daripada enam gen-gen virulen (*iutA*, *hlyA*, *sfa/foc*, *afa/dra*, *kpsMII* dan *pap*) yang dikaji daripada isolat-isolat *E. coli*-ESBL, *iutA* lebih kerap ditemui ($n=56$, 59%) diikuti dengan *kpsMII*

(n=33, 34.7%). Gen-gen yang mengkod untuk *afa/foc* tidak ditemui dalam mana-mana isolat sementara *sfa/foc* dan *hlyA* jarang ditemui. Daripada kesemua isolate-isolat (95) yang dikaji, 27 (28%) tidak membawa apa-apa factor virulen. Analisa perbezaan di antara kumpulan-kumpulan patogenik dan komensal bagi *iutA* tidak menunjukkan hubungkait ketara ($P>0.05$) sementara *kpsMIII* pula adalah amat ketara ($P<0.05$).

Secara kesimpulannya, isolat-isolat daripada jenis *CTX-M* yang menghasilkan strain virulen *E. coli* dari kalangan kumpulan komensal yang didapati daripada jangkitan-jangkitan luar usus menimbulkan keperluan segera dalam mengawal patogen-patogen sebegini kerana ianya mendatangkan potensi untuk menyebarkan rintangan antibiotik dan sifat virulen kepada patogen-patogen dari persekitaran hospital.

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This thesis was submitted to Senate of Universiti Putra Malaysia and has been accepted as fulfilment of requirement for degree of Master of Science. 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 acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

**KHADEGA YAHYA ABDULLAH AL-
HETAR**

Date: 25 May 2011

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