



UNIVERSITI PUTRA MALAYSIA

**MOLECULAR CHARACTERIZATION OF ESBL PRODUCING
KLEBSIELLA SPECIES ISOLATED FROM SEVERAL MAJOR
HOSPITALS IN IRAN**

SOBHAN GHAFOURIAN

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By

SOBHAN GHAFOURIAN

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

July 2011

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Chairman: Associate Professor Zamberi Bin Sekawi, PhD

Faculty: Medicine and Health Sciences

Extended Sepectrum beta-lactamas have been found in a wide range of Gram-negative rods. However, the vast majority of strains expressing these enzymes belong to the *Enterobacteriaceae* family. *K.pneumoniae* remains as the major ESBL producer. The strong selective pressure for the use of beta-lactam drugs exerted on ESBL producer strains may lead to the selection of strains that hyper produce ESBLs. The plasmids that harbor genes encoding ESBLs frequently contain other genes encoding mechanisms of resistance to aminoglycoside and cotrimoxazole. Over the last two decades, the incidence of infections caused by multidrug-resistant *Klebsiella* strains has increased. Extended spectrum beta-lactamase enzymes were first described in *K. pneumoniae* isolates in 1983 in Europe. The focus of this study was To determine epidemiology of ESBL-producing *K. pneumoniae* and *K. oxytoca* in Iran during different seasons, To identify the prevalence of ESBLs producing *K. pneumoniae* and *K. oxytoca* in Iran during different seasons. To determine the prevalence of blaTEM, SHV and CTX-M responsible for ESBL production among ESBL-producing *K. pneumoniae* and *K. oxytoca* in the different wards and hospitals

in Iran. To investigate the susceptibility of *K. pneumoniae* and *K. oxytoca* producing ESBLs towards non beta- lactam antibiotics. To identify the various clonal types of ESBL-producing *K. pneumoniae* in Milad hospital. To detect the dominant ESBL clonal types. Six hundred and seven *Klebsiella spp* were identified during the period March 2007 to April 2008 in three hospitals in three cities (Ilam, Tabriz and Tehran) in Iran. The strains were isolated from urinary tract infections, Intensive care units, surgery wards, lesion infections and Respiratory tract infections. ESBLs were identified by phenotypic and genotypic methods. *Klebsiella spp* producing ESBLs were evaluated against non beta- lactam antibiotics. MLST was performed for dissemination of ESBL producing *K. pneumoniae*. Of the six hundred and seven *Klebsiella spp* isolated from the three hospitals, 34.26%, 16.96% and 43.65% *K. pneumoniae* were obtained from Ilam, Emam Reza and Milad hospitals, respectively. Further, 1.98%, 0.66% and 2.47% *Klebsiella oxytoca* were also obtained from Ilam, Emam Reza and Milad hospitals, respectively. The findings in this study revealed that 36.5%, 51.7% and 45.6% of *K.pneumoniae* were producing ESBLs in Ilam, Milad and Emam Reza hospitals, respectively. The highest ESBLs production of *K.pneumoniae* observed in winter in RTI (54.5%). As for *K.oxytoca* it showed that 25%, 73.3% and 75% of the isolates were positive for ESBLs production in Ilam, Milad and Emam Reza hospitals, respectively. The most *K.oxytoca* and ESBLs producing *K.oxytoca* recurred in winter. Resistance towards non-beta-lactam antibiotics in *K. oxytoca* was only observed in Milad hospital and found in cotrimoxazol and amikacin. In Ilam hospital, of the seventy-six *K.pneumoniae* producing ESBLs, 9.21% were resistant to amikacin, 3.94% to ciprofloxacin and 11.74%, to cotrimoxazol. Of the one hundred and thirty seven *K.pneumoniae* producing ESBLs in Milad hospital, 35.8%, 21.2% and 38.7% of them were resistant

to amikacin, ciprofloxacin and cotrimoxazol, respectively. Resistance toward all the antibiotic in this study in cold seasons was more than the other seasons. In Emam Reza hospital, 21.2%, 4.25%, 21.2% and 0% of *K.pneumoniae* producing ESBLs showed resistance to amikacin, ciprofloxacin, cotrimoxazol and imipenem, respectively. In all the *K.oxytoca*, blaSHV was responsible for the production of ESBLs. Thirty-five blaTEM, two hundred and eighteen blaSHV and fifty-six blaCTX-M were responsible for ESBLs production in *K.pneumoniae*. The analysis showed significant difference of ESBLs production by *K.pneumoniae* in winter (53%) in comparison to the other seasons with $P \leq 0.01$. *K.pneumoniae* producing ESBLs more detected in RTI with $P \leq 0.03$. The results also showed significance different in to blaSHV that was dominant gene responsible for ESBLs production $P \leq 0.049$ but no significant difference observed in blaTEM and blaSHV.

Based on the nucleotide variations of the five selected genetic loci, twenty-five different STs could be identified among thirty *K.pneumoniae* producing ESBLs isolates. The most frequently encountered were ST14 (four isolates) ST16 (two isolates) and ST18 (two isolates). Six clonal complexes were also identified. This study, conducted in different seasons and on different wards, is the first of its kind in the world. The prevalence of ESBLs among clinical isolates varied in different hospital in Iran, the highest prevalence was observed in Milad hospitals (51.6%) follow by Emam Reza (43.7%) and Ilam hospitals (36.5%). Generally, the findings released more prevalence of ESBLs production in Iran. The results showed that the highest ESBLs production was found in *K.oxytoca* isolated from patients in Emam Reza Hospital, Tabriz, and the lowest frequency of ESBLs production was found in *K.oxytoca* in Ilam hospital. BlaSHV was found as dominant gene responsible for ESBLs production by *K. pneumoniae* and *K.oxytoca* and followed by blaCTX-M.

Different clonal complex and ST obtained. CC1 and ST14 were found as a dominant CC and ST, respectively. ESBL-producing isolates in this study were found to be concomitantly resistant to various antibiotic classes, indicating the co-transfer of a range of genes accounting for resistance to these antibiotics. Therefore, therapeutic choices became limited in our hospital. Based on our in vitro findings, imipenem was the most effective antibiotic against ESBL-producing *K. pneumoniae*, followed by Ciprofloxacin.

Key Words: ESBLs, *Klebsiella spp*, MLST, Iran



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PENCIRIAN MOLEKUL BAGI SPESIES KLEBSIELLA YANG MENGHASILKAN ESBL YANG DIPENCILKAN DARIPADA BEBERAPA HOSPITAL YANG UTAMA DI IRAN

Oleh

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Extended Spektrum beta-laktamase telah dijumpai dalam pelbagai rod Gram-negatif. Walau bagaimanapun, majoriti strain yang mengeluarkan enzim ini terdiri daripada keluarga Enterobacteriaceae. *K. pneumoniae* kekal sebagai pengeluar ESBL utama. Tekanan selektif yang kuat ke atas strain pengeluar ESBL dalam penggunaan antibiotik beta-laktam boleh menyebabkan pemilihan strain yang menghasilkan ESBLs secara berlebihan. Plasmids yang membawa gen pengekodan ESBLs sering mengandungi gen pengekodan mekanisme rintangan aminoglycoside dan cotrimoxazole. Sepanjang dua dekad yang lalu, insiden jangkitan yang disebabkan oleh Klebsiella strain rintangan pelbagai telah meningkat. Extended spektrum enzim beta-laktamase telah mula diterangkan dalam *K. pneumoniae* diasingkan pada tahun 1983 di Eropah. Penyelidikan yang dijalankan adalah bertujuan untuk mengkaji epidemiologi molekul bagi bakteria *Klebsiella* ssp. (yang menghasilkan ESBLs) di kalangan pesakit yang dirawat di hospital yang tertentu di negara Iran, untuk mengkaji keberkesanan *Klebsiella* ssp (yang menghasilkan ESBL) terhadap pelbagai jenis antibiotik (jenis bukan β -laktam) pada musim yang berbeza, untuk mengenal pasti pelbagai jenis klon *Klebsiella pneumoniae* (yang menghasilkan ESBL) dari hospital Milad dan untuk menentukan jenis klon ESBL yang dominan. Enam ratus

tujuh jenis *Klebsiella* ssp telah dipencil dan dikenal pasti dari lima hospital bagi tiga bandar yang berbeza (Ilam, Tabriz dan Tehran) di Iran bagi tempoh yang bermula dari Mac 2007 sehingga April 2008. Strain-strain tersebut telah dipencilkan daripada pesakit-pesakit yang menghadapi jangkitan UTI, pesakit di wad ICU, wad pembedahan, pesakit dengan jangkitan yang disebabkan luka dan RTI. ESBLs telah dikenal pasti melalui kaedah fenotip dan genotip. *Klebsiella* ssp (yang menghasilkan ESBLs) telah diuji terhadap antibiotik- antibiotik (jenis bukan β -laktam). Ujian MLST telah dijalankan bagi menentukan sebaran bagi spesies *K. pneumoniae* (yang menghasilkan ESBLs). Daripada 607 *Klebsiella* ssp yang dipencilkan, 34.26%, 16.96% dan 43.65% dengan masing-masing dikenal pasti dan diperoleh dari hospital Ilam, Emam Reza dan Milad. Bagi spesies *K. oxytoca* pula, angka peratusan adalah 1.98%, 0.66% dan 2.47% dengan masing-masing juga dari hospital Ilam, Emam Reza dan Milad. Hasil penemuan daripada kajian ini juga menunjukkan bahawa isolat *K. pneumoniae* yang diperoleh dari hospital Ilam, Milad dan Emam Reza adalah dari jenis yang menghasilkan ESBL dengan masing-masing 36.5%, 51.7% dan 45.6%. Seterusnya dari hospital-hospital yang sama juga (Ilam, Milad dan Emam Reza), penghasilan. Pengeluaran ESBLs tertinggi daripada *K.pneumoniae* diperhatikan dalam musim sejuk di RTi (54.5%). ESBL adalah positif bagi isolate *K. oxytoca* dengan masing-masing mencatatkan peratusan sebanyak 25%, 73.3% dan 75% di Ilam, bathil dan hospital Emam Reza, masing-masing. *K.oxytoca* yang paling dan ESBLs menghasilkan *K.oxytoca* berulang pada musim sejuk.. Kerintangan terhadap antibiotik dari jenis bukan- β – laktam (cotrimoxazol dan amikacin) hanya didapati daripada isolate yang diperoleh daripada hospital Milad. Daripada 76 isolat *K. pneumoniae* (yang menghasilkan ESBLs) yang diperoleh dari hospital Ilam, peratusannya adalah 9.21%, 3.94% dan 11.74% dengan masing-masing rintang terhadap antibiotic

amikacin, ciprofloxacin dan cotrimoxazol. Bagi hospital Milad pula, sebanyak 137 isolat *K. pneumoniae* (yang menghasilkan ESBLs) telah diperoleh dengan peratus rintang sebanyak 35.8%, 21.2% dan 38.7% masing-masing bagi amikacin, ciprofloxacin dan cotrimoxazol. Kerintangan terhadap semua antibiotik dalam kajian ini, pada musim sejuk adalah lebih daripada musim yang lain. Berlainan pula dengan kerintangan antibiotik yang ditunjukkan oleh isolat *K.pneumoniae* (yang menghasilkan ESBLs) dari hospital Emam Reza yang mencatatkan 21.2%, 4.25%, 21.2% dan 0% dengan masing-masing adalah amikacin, ciprofloxacin, cotrimoxazol dan imipenem. Analisis menunjukkan perbezaan ketara pengeluaran ESBLs oleh *K. pneumoniae* pada musim sejuk (53%) berbanding dengan musim lain dengan $P \leq 0,01$. *K. pneumoniae* menghasilkan ESBLs lebih kerap dikesan pada pesakit RTI dengan $P \leq 0.03$. Keputusan juga menunjukkan perbezaan yang signifikan untuk blaSHV sebagai gen dominan yang bertanggungjawab bagi pengeluaran ESBLs ($P \leq 0.049$) tetapi tiada perbezaan yang signifikan yang diperhatikan dalam blaTEM dan blaSHV. Gen blaSHV berperanan penting untuk penghasilan ESBLs bagi *K.oxytoca*. manakala gen blaTEM, blaSHV dan blaCTX-M pula bertanggungjawab untuk penghasilan ESBLs bagi *K. pneumoniae* dengan masing-masing adalah 35, 218 dan 56. Berdasarkan kepada variasi genetik bagi 5 lokus genetik, 25 STs yang berbeza telah dikenal pasti daripada 30 isolat *K. pneumoniae* (yang menghasilkan ESBLs). Kekerapan yang tinggi adalah bagi strain ST14 iaitu sebanyak 4 isolat, 2 isolat bagi ST16 dan 2 isolat bagi ST18. Prevalens ESBLs di kalangan isolat klinikal yang berbeza-beza di hospital yang berlainan di Iran, prevalens tertinggi diperhatikan di hospital-hospital Milad (51.6%) diikuti dengan Emam Reza (43.7%) dan hospital Ilam (36.5%). Secara amnya, penemuan ini mengesahkan prevalens ESBLs di Iran. BlaSHV didapati sebagai gen dominan yang bertanggungjawab bagi pengeluaran

ESBLs oleh *K. pneumoniae* dan *K. oxytoca* dan diikuti oleh blaCTX-M. Kompleks klonal yang berbeza dan ST diperolehi. CC1 dan ST14 ditemui sebagai CC dominan dan ST masing-masing. Isolat ESBL dalam kajian ini didapati rintang kepada pelbagai kelas antibiotik, menunjukkan bersama pemindahan pelbagai gen yang menyumbang untuk rintangan antibiotik ini. Oleh itu, pilihan terapeutik menjadi terhad di hospital kami. Berdasarkan dalam penemuan in vitro, imipenem antibiotik yang paling berkesan terhadap ESBL menghasilkan *K. pneumoniae*, yang diikuti oleh Ciprofloxacin.

Kata Kunci: ESBLs, *Klebsiella* spp, MLST, Iran

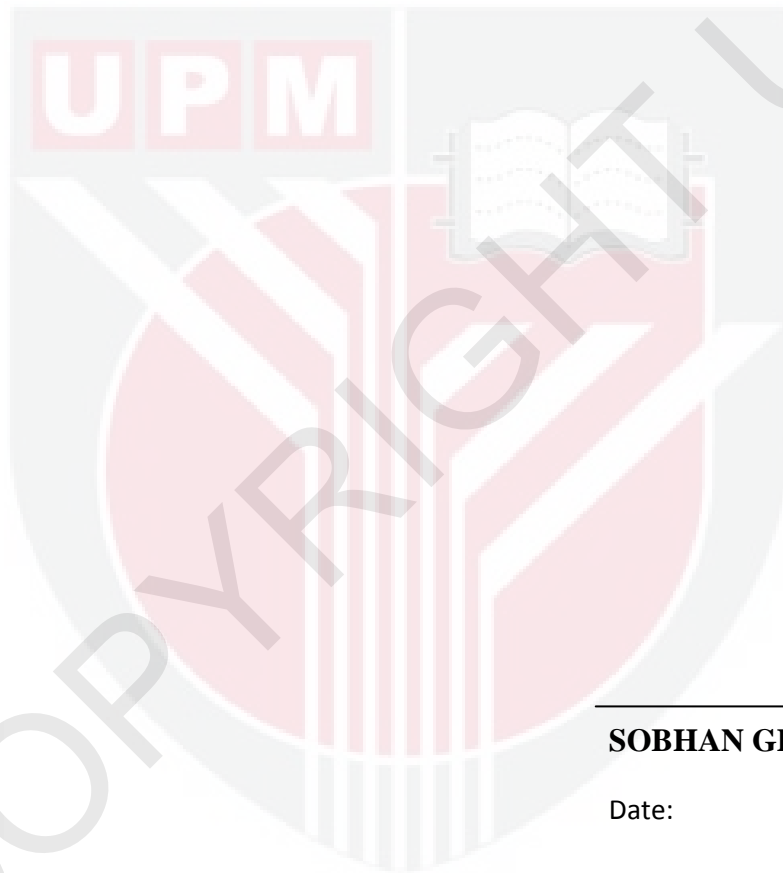
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DECLARATION

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 concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



SOBHAN GHAFOURIAN

Date:

I certify that an Examination Committee has met on 22/07/2011 to conduct the final examination of sobhan Ghafourian on his Master of Sciences thesis entitled 'Molecular characterization of ESBL producing *Klebsiella* species isolated from several major hospitals in Iran'' in accordance with University Putra Malaysia (Higher Degree) and Universiti Pertanian Malaysia (Higher Degree) Regulations. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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