Phenotypic Characterization of Extended Spectrum Beta Lactamase in Clinical Isolate of Klebsiella Species Isolated In Intensive Care Unit

Thesis

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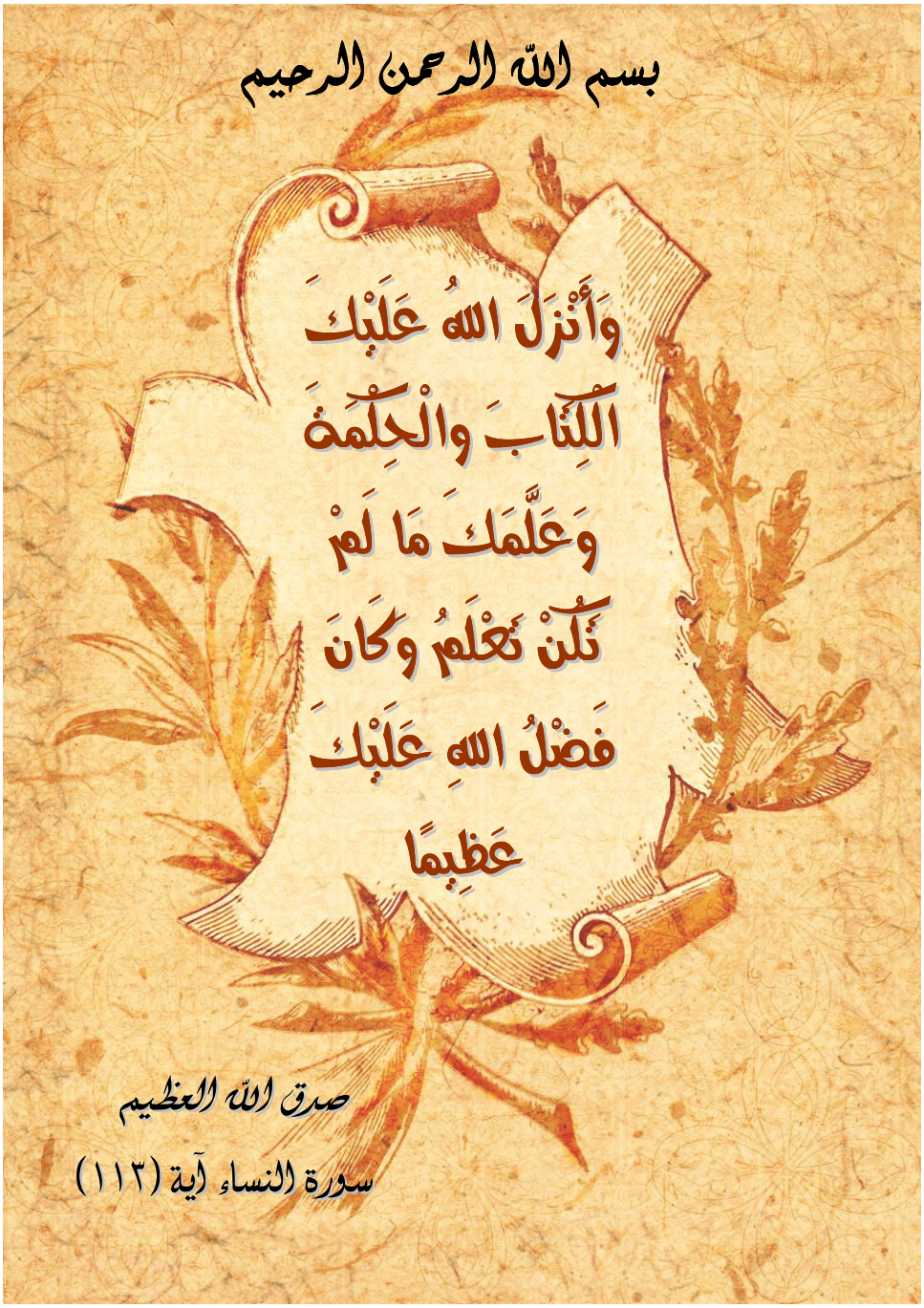
بسم الله الرحمن الرحيم
وَأَنْزَلَ اللهُ عَلَيْكَ
الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ
تَكُنْ تَعْلَمُ وَكَانَ
فَضْلُ اللهِ عَلَيْكَ
عَظِيمًا
صدق الله العظيم
سورة النساء آية (١١٣)

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Thank you all for being wonderful

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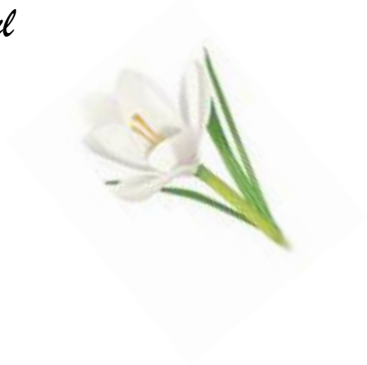

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List of Abbreviations

Abb.	Full term
ACT-1	AmpC resistance in association with ESBL production
AMC	Amoxicillin/Clavulanate
AmpC	Ambler class C enzymes
Arg	Arginine residue
ASC	Active surveillance cultures
Asp	Aspartine residue
BSAC	British Society for Antimicrobial Chemotherapy
CAMHB	Cation adjusted Muller-Hinton broth
CAZ	Ceftazidime
CAZ/CLA	Ceftazidime in combination with clavulanate
CLSI	The Clinical Laboratory Standards Institute
CMT-1 to 4	Complex Mutants of TEM-1 to 4
CPD	Cefpodoxime
CRO	Ceftriaxone
CT	Cefotaxime in E-test
CTL	Cefotaxime with clavulanate in E-test
CTX	Cefotaxime
CTX/CLA	Cefotaxime in combination with clavulanate
DD	Double Disk test
DDS	Double disk synergy
E.coli	*Escherichia coli*

EARSS	European Antibiotic Resistance Surveillance System
ESBL	Extended-spectrum β-lactamase
ESBL-E	Extended-spectrumβ-lactamase-producing *E.coli*
ESBL-EK	Extended-spectrumβ-lactamase-producing *E.coli* and *klebsiella* spp.
ESBL-K	Extended-spectrumβ-lactamase-producing *klebsiella* spp.
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
FEP	Cefepime
Glu	Glucine residue
Gly	Glycine residue
GNB	Gram negative bacilli
His	Histadine residue
ICU	Intensive care units
IDSA	Infectious Diseases Society of America
K.pneumoniae	*Klebsiella pneumoniae*
LIA	Lysine Iron Agar
Lys	Lysine residue
MBLs	Metallo β-lactamases
MDR	Multi drug resistance
MDRO	Multi drug resistance organisms
MIC	Minimal inhibitory concentration
MYSTIC	Meropenem Yearly Susceptibility Test Information Collection
P.mirabilis	*Proteus mirabilis*
PCR	Polymerase chain reaction

PM	Cefepime in E-test
PML	Cefepime with clavulanate in E-test
Ser	Serine residue
SFM	Sociėtė Française de Microbiologie
Spp	Species
Thr	Theronine residue
TSB	Tryptone soya broth
TSI	Triple Sugar Iron Agar
TZ	Cetazidime in E-test
TZL	Cetazidime with clavulanate in E-test
UTI	Urinary tract infection
VAP	Ventilator associated pneumonia

INTRODUCTION

Multi-drug resistant gram negative bacilli belonging to the family enterobactericae have been increasingly responsible for many types of infection in hospitals in many countries ***(Rashid, 2010).***

Klebsiella spp. constitutes the majority of these pathogens. Resistance to klebsialla pneumonaie to extended spectrum beta lactmase antibiotics is commonly mediated by B. lactamases (ESBL) ***(Vinue et al., 2008)***.

ESBLs are calvulonate susceptible enzymes capable of hydrolyzing oxi-imino cephalosporins and monobactams but not cephamycins & carbapenems, these enzymes are originally observed in Escherichia coli and klebsiella spp. ESBL production has now been documented in other gram negative bacilli including enterobacter spp., proteus mirabilis and providentia steratii ***(Messai et al., 2008).***

Because infection with ESBL producing organisms are often not adequately covered with empirically given antibiotics, the proper choices of antibiotic therapy and infection control measures depend upon early and accurate ESBL detection, it is therefore pivotal to have a rapid and sensitive laboratory assay ***(Pitout and Laupland, 2008).***

ESBL-produsing micro-organisms exhibit high level of resistance to benzyl penicillin & narrow spectrum

cephalosporins. The MICS for aztreonam and expanded-spectrum, broad spectrum, and fourth generation cephalosporin e.g (cefpiame, cefepime) vary greatly, because the various mutations offer different phenotypic expression ESBL-producing resistance, therefore, posses problem for in vitro susceptibility testing, first because of limited number of cephalosporins routinely used in the laboratory and second because the MIC may not reach the (NCCLS) break points for resistance when the standard inoculum of 105 CFU/ml is used. Nosocomial outbreaks are often caused by ESBL producing isolates, particularly in intensive care unit, they result from clinical transmission of epidemic isolate and/or the horizontal transfer of resistance genes ***(Messai et al., 2008).***

Therefore, it is necessary to use special ESBL detection tests to avoid the risk reporting false susceptibility to penicillins, cephalosporins and aztreonam. Several phenotypic methods has been used for ESBL detection (ESBL H tests, combination discs, double disc synergy test, cloxacillin centering Muller Hinton Agar & cica-Beta test), also semi automated microbiology system have been used for ESBL detection as phoenix automated microbiology system (BD, Diagnostic systems, spartus, MD), VITEK 2 system (Bioreueux Marcy L`Etoile, Enarce) and the Microscan walk away-96 system (Dade behring, Inc, west sacramento,CA) using routine testing panel ***(David et al., 2009).***

Aim of the Study

Evaluation of the ESBL test and double disc synergy test as phenotypic method for detection of ESBL and comparing the results with microscan walk away-96 system in ESBL detection in klebsiella spp. isolated from intensive care unit.

Chapter (1)

KLEBSIELLA SPECIES

Klebsiella was described in the mid 1880 and was named after the german microbiologist Edwin Klebs. The bacillus was also described by Carl Friedlander, and for many years the Friedlander bacillus was known as a cause of fatal pneumonia. However, for almost 70 years microbiologists were unable to separate Klebsiella from Aerobacter aerogenes ***(Grimont et al., 1992).***

Taxonomy

In 1971, Bascomb and colleagues used numerical taxonomy as a basis for calassifying klebsiella into six taxa. They retained k.pneumoniae, k.ozaenae, and k.rhinoscleromatis as separate groups whereas k.aerogenes, k.edwardsii and k.oxytoca were placed in a single taxon. Enterobacter aerogenes was included as a motile species of klebsiella named k.mobilis. Also, another group that remained unnamed was established. The introduction of DNA hybridization showed the limited relatedness between E.aerogenes and klebsiella. Thus researchers transfer E.aerogenes to new genus Enterobacter ***(Brenner et al., 1972).***

Furthermore, the DNA data showed that K.ozaenae, K.edwardsii, and K.rhinoscleromatis could not be separate from K.pneumoniae ***(O'rskov, 1984).***

In 1981, the environmental species, K.planticola and K.terrigena, were added to the genus Klebsiella. They were originally called group K or K.trevisani and group L (for K.planticola and K.terrigena) respectively ***(Bagley et al., 1981).***

K.lebsiella ornitholytica was the last organism to be added to the genus Klebsiella. This is the only species of Klebsiella that decarboxylates ornithine. It was formerly known as ornithine-positive K.oxytoca or as the enteric group ***(Janda at al., 1997).***

Pathogenesis and virulence factors

Recent data from preclinical studies suggest a role for neutrophil myeloperoxidase and lipopolysaccharide-binding protein in host defense against K pneumoniae infection. Neutrophil myeloperoxidase is thought to mediate oxidative inactivation of elastase, an enzyme implicated in the pathogenesis of various tissue-destroying diseases. Lipopolysaccharide-binding protein facilitates transfer of bacterial cell wall components to inflammatory cells ***(Won et al., 2011).***