Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Phenotypic and genotypic studies on antimicrobial resistance of lower respiratory tract bacterial pathogens

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences
(Microbiology and Immunology)

By

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والحمد لله رب العالمين.....

Salma Mustafa Abdelaziz

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List of Abbreviations

AAC(6')-Ib	aminoglycoside 6'-N-acetyltransferase type Ib enzyme
aac(6')-Ib	Gene coding for aminoglycoside 6'-N-acetyltransferase type Ib
aac(6')-Ib-cr	Gene coding for aminoglycoside 6'-N-acetyltransferase type Ib ciprofloxacin resistant variant
AC	Accession code
API	Analytical profile index
ATS	American Thoracic Society
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CAP	Community-acquired pneumonia
cfu	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CTX-M	CTX-M extended spectrum β-lactamase enzyme
ctx-m	Gene coding for CTX-M extended spectrum β-lactamase
DDST	Double disc synergy test
EDTA	Ethylene diaminetetraacetic acid
EMB	Eosin methylene blue
ESBL(s)	Extended spectrum β-lactamase(s)
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
LB	Luria Bertani

LRTI(s)	Lower respiratory tract infection(s)
MDR	Multiple drug resistant
MH	Mueller-Hinton
MIC	Minimum inhibitory concentration
NCBI	National Center for Biotechnology Information
OMP	Outer membrane protein
ORF	Open reading frame
PBP(s)	Penicillin binding protein(s)
PCR	Polymerase chain reaction
PER	*Pseudomonas* extended resistance
qnr	Gene coding for quinolone resistance
rpm	Round per minute
RTI(s)	Respiratory tract infection(s)
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulphate
SHV	SHV extended spectrum β-lactamase enzyme
shv	Gene coding for SHV extended spectrum β-lactamase
SOB	Super optimal broth
SOC	Super optimal broth with catabolite repression
T_a	Annealing temperature
TAE	Tris-acetic acid-EDTA
TE	Tris-EDTA
TEM	TEM extended spectrum β-lactamase enzyme

tem	Gene coding for TEM extended spectrum β-lactamase
T_m	Melting temperature
Tris	Trishydroxymethylaminomethane
tRNA	Transfer ribonucleic acid
TSI	Triple sugar iron agar
URTI(s)	Upper respiratory tract infection(s)
VEB	Vietnamese extended spectrum β-lactamase

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Abstract

This study involved the recovery of 235 bacterial isolates from sputum and bronchoalveolar lavage specimens of lower respiratory tract infected patients. Using Gram-stain, 118 isolates (50.2%) were Gram-positive and 117 isolates (49.8%) were Gram-negative. The susceptibilities of the recovered isolates were tested to sixteen different antimicrobial agents commonly used to treat lower respiratory tract infections. Results revealed that the lowest resistance was observed to amikacin, doxycycline and meropenem; where 19 isolates (8%) were resistant to each. While the highest resistance was observed to clindamycin; 108 isolates (45.9%) were resistant. In case of Gram-positive bacterial isolates, the lowest resistance was observed to doxycycline; where only one isolate (0.8%) was resistant. On the other hand, the highest resistance was observed to clindamycin and erythromycin where 20 isolates (16.9%) showed resistance for each of them. For Gram-negative bacterial isolates, the lowest resistance was observed to meropenem and azithromycin; only 13 isolates (11.1%) were resistant to each. The highest resistance was observed to clindamycin; 88 isolates (75.2%) were resistant. Among the 235 collected isolates; 62 isolates (26.4%) were found to be resistant to members belonging to three or more classes of antimicrobial agents; 53 isolates were Gram-negative (85.5%) while 9 isolates were Gram-positive (14.5%). Out of these 62 multiple drug resistant (MDR) isolates; 23 isolates were *Klebsiella pneumoniae* (37.1%), 15 isolates were *Escherichia coli* (24.2%), 10 isolates were *Pseudomonas aeruginosa* (16.1%), 9 isolates were *Staphylococcus aureus* (14.5%), 3 isolates were *Enterobacter cloacae* (4.8%) and 2 isolates were *Acinetobacter baumannii* (3.2%).

Plasmid bands were detected in 60 (96.8%) out of the 62 MDR isolates which included 7 *S. aureus* isolates (77.8%) and all the tested Gram-negative bacterial isolates (n=53; 100%). The nineteen isolates resistant to amikacin were found to carry the aminoglycoside acetyltransferase gene (*aac6'-Ib)* reported to be