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Susceptibility Pattern, Genotyping, and Mutations of *Klebsiella pneumoniae* at Dr. H. Abdul Moeloek General Hospital

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ABSTRACT

Klebsiella pneumoniae is one of the most common causes of severe hospital-acquired infection. ESBL-producing *Klebsiella pneumoniae* causes a major problem for clinical management and epidemiological study. The other factor identified was OmpK35 which is often poorly or not expressed and it can be altered by factors such as point mutations. This study aimed to determine the susceptibility pattern, and the genotyping and to investigate the mutations in OmpK35 of *Klebsiella pneumoniae*. This is a cross-sectional study using susceptibility pattern data from the ninety isolates of *Klebsiella pneumoniae* from the patients admitted to Dr. H. Abdul Moeloek General Hospital, Lampung. The Genotype of ESBL genes and OmpK35 gene were determined by polymerase chain reaction and sequencing for identification of the mutation. The susceptibility rate of *Klebsiella pneumoniae* belonged to Ampicillin was 0%. The susceptibility rate belonged to Amikacin (96.6%), Meropenem (94.4%), and Ertapenem (94.4%). From 90 isolates, the genotype blaSHV was found in 86.7%, and most of the isolates had OmpK35 genes (91.2%). Among the thirty isolates, 20% harbored mutations in the OmpK35 protein with substitution mutations. This finding indicated a high prevalence of antibiotic resistance, a high prevalence rate of ESBL gene production, and a high frequency of porin mutations among *Klebsiella pneumoniae* isolates.

Keywords: *Klebsiella pneumoniae*, susceptibility pattern, genotyping, mutations

INTRODUCTION

The emergence of Extensively Drug-Resistant (XDR) *Klebsiella pneumoniae* poses a significant global concern. This bacteria is a major human pathogen and is implicated in opportunistic nosocomial and community-acquired infections.[1,2] While *Klebsiella pneumoniae* is typically found in the gastrointestinal flora, it can also cause severe infections like urinary tract infections, pneumonia, and life-threatening bacteremia, resulting in high rates of illness and death.[3] Moreover, the rise of XDR strains of *Klebsiella. pneumoniae* exerts significant pressure on healthcare systems, leading to severe nosocomial infections.[4,5]

The global incidence of ESBL in *Klebsiella pneumoniae* is progressively rising. The global data showed that the frequency of ESBL in *Klebsiella pneumoniae* was 33.3% in Iran, 58% in India, 38.6% in Taiwan, and 52.9% in Indonesia.[6-8] ESBL production is one of the resistance mechanisms of the Klebsiella genus and primarily arises due to mutations in beta-lactamases. Mutations encoded by genes such as blaSHV, blaTEM, and blaCTX. These genes, which are frequently detected in *Klebsiella pneumoniae*, exhibit variations in their prevalence among hospital isolates, depending on the geographical region.[7-9]

The outer membrane of Gram-negative bacteria is a complex structure that plays a crucial role in various functions. A family of proteins called Outer Membrane Proteins (Omp) or porins, whose expression in the outer membrane varies depending on the environmental conditions of the host.[10] These porins have been identified as recently discovered virulence factors. In the case of *Klebsiella pneumoniae*, the main porins are OmpK35 and OmpK36.[11,12] Porins play a crucial role in bacterial survival by facilitating the exchange of substances, including nutrients and toxic metabolites. These porins are also instrumental in the passage of antibiotics into the bacterial cell.[12]

In the *Klebsiella pneumoniae*, the expression of porins can be modified by various factors, such as point mutations or interruptions in the coding sequences or promoter region. The lack of both OmpK35 and OmpK36 can cause antibiotic resistance.[12,13] Among ESBL-producing *Klebsiella pneumoniae* strains, a majority lack OmpK35. The absence of this specific porin in ESBL-producing *Klebsiella pneumoniae* strains can contribute to

antimicrobial resistance and potentially enhance the selection of additional resistance mechanisms, such as the loss of OmpK36 and/or active efflux.[10,12,13] The objective of this study is to assess the antibiotic resistance pattern, and the gene type of ESBL, and investigate the mutations occurring in the OmpK35 porin of *Klebsiella pneumoniae.*

METHODS

The ninety isolates of *Klebsiella pneumoniae*, including blood, sputum, pus, wound swab, urine, and body fluids were identified and tested for antibiotic susceptibility using the Vitek® 2 compact instrument at the Microbiology Laboratory.Dr. H. Abdul Moeloek Hospital, Lampung. The minimum inhibitory concentration (MIC) values were interpreted as susceptible (S), intermediate (I), or resistant (R) based on the criteria set by the Clinical Laboratory Standards Institute (CLSI). ESBL production was evaluated in strains showing decreased susceptibility to third-generation Cephalosporins using the Vitek® 2 system. A confirmatory test for phenotypic detection of ESBLs was performed using the Double Disk Synergy Test (DDST) method.

The isolates underwent additional analysis through PCR to identify beta-lactamase genes. PCR amplification was performed using a thermal cycler with two sets of primers designed to target different regions and detect blaTEM, blaSHV, and blaCTX genes. The resulting PCR products were separated via gel electrophoresis on a 1% agarose gel. Polymerase Chain Reaction (PCR) was conducted using two sets of primers designed to target different regions for the detection of the OmpK35 encoding gene. The PCR products, along with a DNA ladder, were separated using an electrophoresis system.

The.25 μLPCR products of 30 bacterial samples resistant to OmpK35 gene analysis, along with the corresponding primers, were sent to Macrogen Company (Singapore) for sequencing the plus strand only. The software MEGA (Molecular Evolution Genetics Analysis) X was utilized for the detection of mutations in this gene. This study, used the two American Type Culture Collection, USA (ATCC) strains *Klebsiella pneumoniae*. ATCC 13883 is the negative control and ATCC 700603 is the ESBL positive control. Statistical analysis using SPSS software version 20 and the Chi-Square test was used for data analysis. Ethical Clearance by the Ethical Committee of the Dr. H. Abdul Moeloek General Hospital, Lampung, approved the study protocol No. 003/KEPK-RSUDAM /VI/2023.

RESULTS AND DISCUSSIONS

Klebsiella pneumoniae is a major pathogenic bacterium responsible for a range of clinical conditions, such as urinary tract infections, pneumonia, skin and soft tissue infections, as well as bacteremia and septicemia.[14,15] In this study, a total of 90 K*lebsiella pneumoniae* isolates were obtained from different sources, including pus (46.1%), sputum (15.3%), blood (15.3%), wound swab (10.9%), urine (8.79%), and body fluids (3.29%).

From Table 1, the majority of isolates displayed resistance to extended-spectrum third-generation Cephalosporins. The most effective antibiotics were Amikacin, with a susceptibility rate of 96.6%, followed by Meropenem and Ertapenem, both with a rate of 94.4% each. These findings are consistent with the

Table 1. Anti microbial susceptibility patterns of *Klebsiella pneumoniae* (n=90)

Class	Antibiotics	Susceptibility Rate (%)
Penicillin	Amoxicillin	8 (8.8%)
	Ampicillin	0
B-lactam inhibitor	Sulbactam Amoxicillin	37 (41.2%)
	Tazobactam Piperacillin	64 (71.2%)
Cephalosporin	Cefazoline	41 (45.6%)
	Cefotaxime	40 (43.4%)
	Ceftazidime	38 (42.3%)
	Ceftriaxone	39 (43.4%)
	Cefepime	39 (43.4%)
Monobactam	Aztreonam	39 (43.4%)
Carbapenem	Ertapenem	85 (94.4%)
	Meropenem	85 (94.4%)
Aminoglycoside	Amikacin	87 (96.6%)
	Gentamicin	59 (65.5%)
Quinolone	Ciprofloxacin	60 (66.7%)

studies conducted by Kaur *et al*. and Wulandhany *et al*., which reported a high prevalence of resistance. *Klebsiella pneumoniae* strains to multiple drugs. Specifically, Ampicillin, Cefazolin, and Cefuroxime exhibited the lowest effectiveness, while Amikacin, Piperacillin-Tazobactam, and Meropenem showed the most favorable profiles.[16,17] This observation aligns with the results reported by Jalal *et al*., where Ampicillin displayed the highest resistance rate (97.6%), while Tigecycline exhibited the lowest resistance rate (15.7%).[18]

The emergence of antibiotic resistance is attributed to various factors, including the widespread use of antibiotics in healthcare settings, and communities, as well as in animal production, agriculture, and the environment. Easy accessibility to antibiotics without a prescription contributes to their extensive utilization. Prolonged and excessive antibiotic usage in healthcare settings is considered a major contributing factor to the dissemination of challenging-to-treat antibiotic-resistant nosocomial infections.[19] Nagid, reported a similar overall susceptibility pattern for *Klebsiella pneumoniae* isolates, with high resistance observed against Ampicillin, Ceftriaxone, and Cefepime, while the highest susceptibility rates were observed for Ertapenem and Imipenem.[15] This finding is consistent with the study by Virawan, which found that *Klebsiella pneumoniae* with high sensitivity to Meropenem (98.43%) and Amikacin (93.75%), while being completely resistant to Ampicillin (100%).[20]

This could be attributed to the frequent utilization of this particular antibiotic for treating infections caused by *Enterobacteriaceae*, including *Klebsiella pneumoniae*. Furthermore, the isolates demonstrated a relatively high susceptibility rate to aminoglycosides, with Amikacin showing a resistance rate of 96.6% and Gentamicin at 65.6%. In contrast, Carbapenems (Meropenem and Ertapenem) exhibited higher effectiveness compared to Penicillin and Cephalosporins, as only 5.6% ofthe isolates displayed resistance to them.[16,18] The prevalence of highly drug-resistant. *Klebsiella pneumoniae* has emerged as a significant challenges in health care systems worldwide, posing major clinical concerns. Especially for the resistance to third-generation Cephalosporins and Carbapenems, as β-lactam antibiotics are commonly prescribed. The availability of alternative drugs with comparable safety profiles and clinical efficacy is limited.[19]

Specific strains of *Klebsiella pneumoniae* have acquired the capacity to survive and counteract the impact of beta-lactam antibiotics by generating ESBL enzymes. These enzymes can hydrolyze and render beta-lactam antibiotics, such as Penicillin, first, second, and third-generation Cephalosporins, and Aztreonam ineffective, while Cephamycin or Carbapenems remain unaffected.[18]

In this study, found the gen types of blaTEM, blaSHV, and blaCTX were 86.6%, 60.0%, and 5.5%, respectively. The results were different from Rouf's study *et al*., where 47.4% of blaCTX and 15.8 blaSHV. In the other study by Mohammed *et al*., blaSHV was dominant (92.85%).[21,22]

From Table 2, it is evident that some isolates exhibit the presence of multiple gene types, such as blaTEM with blaCTX, blaTEM with blaSHV, and blaSHV with blaCTX, indicating a potential correlation between ESBL-producing strains and complex antimicrobial resistance.[22]

Table 2. Distribution of ESBL multiple gene types of *Klebsiella pneumoniae* isolates

Type of multiple gene	No. of Sample	(%)
TEM + CTX	3	3.33
TEM + SHV	19	21.1
CTX + SHV	6	6.66
TEM + CTX + SHV	31	34.4

In this study, the most common combination type of isolates was blaTEM and blaSHV (21.2%). This finding was consistent with the results reported by Raouf *et al*., which indicated that a majority (36,8 %) of the ESBL type able isolates harbored two or more beta-lactamase genes, and these isolates showed a higher frequency of antibiotic resistance compared to those with asingle gene.[22]

From Table 3, the susceptibility pattern of *Klebsiella pneumoniae*-producing ESBL indicates that all ESBL-type genes exhibited higher resistance to Ampicillin and Amoxicillin.

The group of isolates with three ESBL-type genes demonstrated even higher resistance to all antibiotics, particularly Ampicillin (100%) and Amoxicillin (90.3%). However, this study found that the blaSHV gene was the dominant ESBL type (86.6%), contradicting the Southeast Asia region where blaCTX enzymes are typically dominant. This is consistent with another study that found blaSHV as the predominant ESBL type in *Klebsiella pneumoniae* (84.8%), surpassing blaTEM and blaCTX types.[23]

The emergence of extended-spectrum beta-lactamases is primarily attributed to mutations in beta-lactamases encoded by blaSHV, blaTEM, and blaCTX-M genes. While TEM and SHV variants are the most common ESBLs, there has been a recent emergence of strains expressing CTX-M ESBLs in various countries. The blaCTX gene is particularly prevalent in *Enterobacteriaceae*, including

Table 3. Susceptibility Pattern of *Klebsiella pneumoniae* according to genetypes of ESBL

Antibiotics	TEM (n=41)	CTX (n=41)	SHV (n=78)	TEM+CTX (n=3)	TEM+SHV (n=19)	CTX+SHV (n=5)	TEM+SHV+CTX (n=31)
	% Susceptibility						
Amoxycillin	14.9	12.2	23.1	0	21.1	0	9.7
Ampicillin	1.9	0	0	0	15.8	0	0
Sulbactam Amoxycillin	50	26.9	57.7	66.7	94.74	33.4	19.4
Piperacillin Tazobactam	90.75	80.5	89.8	100	100	50	83.88
Cefazoline	37.1	22	47.5	33.4	63.2	33.4	19.4
Cefotaxime	42.6	34.2	47.5	0	68.5	33.4	32.3
Ceftazidime	29.7	22	46.2	0	47.4	33.4	19.4
Ceftriaxone	29.7	22	44.88	0	47.4	33.4	19.4
Cefepime	29.7	22	44.88	0	47.4	33.4	19.4
Aztreonam	29.7	22	44.88	0	47.4	33.4	19.4
Ertapenem	92.6	90.3	93.59	100	100	100	87.1
Meropenem	92.6	90.3	61.6	100	100	100	87.1
Amikacin	100	100	98.72	100	100	100	100
Gentamicin	59.3	66.7	66.7	66.7	94.74	50	35.5
Ciprofloxacin	63	66.7	66.7	66.7	89.48	50	45.2

Escherichia coli and *Klebsiella pneumoniae*.[22,23]

In this study, the distribution of the OmpK35 gene in 90 isolates of *Klebsiella pneumonia* was found to be 91.2%, while only 8.8% of the isolates lacked the ompK35 gene. In *Klebsiella pneumoniae*, there are two major porins, namely OmpK35 and OmpK36. It has been observed that most ESBL-producing strains of *Klebsiella pneumoniae* express only OmpK36, while the majority of non-ESBL-producing strains synthesize both OmpK35 and OmpK36.[23] OmpK35 is considered one of the primary outer membrane porins in *Klebsiella pneumoniae*. These genes play a crucial role in the entry of antibiotics into bacterial cells, as antimicrobial drugs need to penetrate the outer membrane before reaching the periplasm. Particularly for beta-lactams, which are typically hydrophilic and charged, porin channels serve as the main route of penetration.[23]

Porins are essential for the survival of bacteria as they enable the exchange of substances, including nutrients and toxic metabolites. Previous studies have shown that a minority of clinical isolates of ESBL-producing *Klebsiella pneumoniae* do not possess both OmpK35 and OmpK36 porins. Interestingly, it has been observed that strains lacking both porins exhibit elevated levels of antibiotic resistance compared to strains that express one or both of these porins.[12]

This study also investigated the relationship between the OmpK3 gene and the antimicrobial susceptibility of *Klebsiella pneumoniae*. Statistically, the results found that the OmpK35 gene was more significantly associated with resistance to Ciprofloxacin (p=0.013) than others (Table 4). *Klebsiella pneumoniae* exhibits high susceptibility to fluoroquinolones, and the absence of capsule or antigen O did not affect the MICs of three fluoroquinolones with varying hydrophobicity.[24]

In one study, the loss of porins did not lead to increased MICs for any of the three agents tested, including the hydrophilic compound Ciprofloxacin. However, other studies have demonstrated a four-fold increase in the MIC of norfloxacin in porin-deficient mutants like KT5003P, as well as in certain clinical isolates of *Klebsiella pneumoniae* with porin deficiencies. Fluoroquinolone-resistant clinical isolates often possess to poisomerase mutations or exhibit reduced drug accumulation, both of which can contribute to fluoroquinolone resistance. It appears that porin deficiency becomes more significant when the drug exhibits lower intrinsic activity or when other resistance mechanisms coexist within the same bacterial cell, as discussed previously about β-lactams.[24]

From Table 5, can see the sequencing results of 30 isolates, that showed the presence of many types of mutation in the OmpK35 gene.

The expression of porins in clinical isolates of *Klebsiella pneumoniae* can be modified due to various factors, including point mutations or disruptions in the coding sequences or promoter region. Strains that lack both OmpK35 and OmpK36 exhibits elevated levels of antibiotic resistance.[25] In this study, 6 isolates (20%) mutations belonged to

Table 4. The relation between the OmpK35 gene to antimicrobial susceptibility of *Klebsiella pneumoniae*

Antibiotics	Susceptibility of *Klebsiella pneumoniae*		p-value*
	OmpK 35 (+)	OmpK 35 (-)	
Amoxycillin	19 (24.7)	2 (15.4)	0.464
Amoxycillin Sulbactam	43 (55.8)	6 (46.2)	0.516
Piperacillin Tazobactam	68 (88.3)	12 (92.3)	0.672
Cefazolin	37 (48.1)	4 (30.8)	0.247
Cefotaxime	37 (48.1)	3 (23.1)	0.094
Ceftazidime	34 (44.2)	4 (30.8)	0.366
Ceftriaxone	35 (45.5)	4 (30.8)	0.323
Cefepime	35 (45.5)	4 (30.8)	0.323
Aztreonam	35 (45.5)	4 (30.8)	0.323
Ertapenem	72 (93.5)	13 (100)	0.344
Meropenem	72 (93.5)	13 (100)	0.344
Amikacin	76 (98.7)	13 (100)	0.679
Gentamycin	52 (67.5)	9 (69.2)	0.904
Ciprofloxacin	51 (66.2)	13 (100)	0.013

*Chi-Square test significant if p-value ≤ 0.05

Table 5. Many types of mutation in the OmpK35 gene of *Klebsiella pneumoniae* isolates (n=30)

No. of Sample	Type of Antibiotic Resistance	Wild Type of Codon	Mutant Type of Codon	Position of Codon	Change in Amino Acid	Type of Mutation	Name of Mutation
4	AMC, AMP, SAM, KZ, CTX, CAZ, CRO, FEP, AZT, CN	**G**AT	**A**AT	55	D>N	substitution	55 D>N
		ACC	**C**CC	56	T>P	substitution	56 T>P
		GAC	**A**AC	159	D>N	substitution	159 D>N
14	AMC,AMP, KZ, CTX, CAZ, CRO, FEP, AZT	**A**AC	**C**AC	143	N>H	substitution	143 N>H
		GAC	**T**AC	221	D>Y	substitution	221 D>Y
15	AMP, AMC	**G**AA	**A**AA	132	E>K	substitution	132E>K
17	AMC,AMP, SAM,CTX, CAZ, CRO, FEP,AZT, CN,CIP	**G**AA	**A**AA	228	E>K	substitution	228 E>K
		GCG	**T**CG	243	A>S	substitution	243 A>S
		AAC	**G**AC	252	N>D	substitution	252 N>D
22	AMC, AMP,CIP	**G**AA	**A**AA	132	E>K	substitution	132 E>K
26	AMC,AMP,KZ,TZP, CTX,CAZ, CRO, FEP, AZT, ERT, MEM, CN,CIP	**G**CG	**A**CG	243	A>T	substitution	243 A>K

the substitution mutation. All mutations were substitution-type mutations as shown in sample numbers 4, 14, 15, 17, 22, and 26, respectively. There was more than one point mutation as shown in sample numbers 4, 14, and 17, and therefore a high rate of resistance to antibiotics especially for that sample.

The absence of one porin, either OmpK35 or OmpK36, did not have an impact on the MICs of *Klebsiella pneumoniae* strains that lacked other resistance mechanisms. Only when both porins were absent, a reduction in the effectiveness of a few beta-lactam antibiotics was observed. This indicates that multiple mutations are typically required for

Klebsiella pneumoniae to develop resistance against clinically important antimicrobial agents. Unfortunately, this is not commonly observed in most clinical isolates of *Klebsiella pneumoniae*, as they usually produce penicillinase, occasionally express ESBL or AmpC-type enzymes, or have to poisomerase mutations. These factors may contribute to the emergence of clinically relevant antimicrobial resistance.[26]

CONCLUSIONS AND SUGGESTIONS

This research revealed a high prevalence of antibiotic resistance, a high ESBL gene production, and a significant number of porin mutations among *Klebsiella pneumoniae* isolates. That all *Klebsiella pneumoniae* isolates were not sensitive to Ampicillin but 96.6% were sensitive to Amikacin, followed by Meropenem and Ertapenem (94.4%). The most common ESBL gene type among *Klebsiella pneumoniae* isolates was blaSHV (86.7%), followed by blaTEM and blaCTX. This study observed a statistically significant association between the presence of the OmpK35 gene and resistance to Ciprofloxacin (p=0.013) compared to other antibiotics. All observed mutations were substitution mutations, as demonstrated in the samples, and multiple point mutations were detected, which may contribute to the high rate of antibiotic resistance. This finding provides an explanation for the resistance mechanisms observed in *Klebsiella pneumoniae*, which involve various mechanisms.

It is suggested that multiple mutations are necessary for *Klebsiella pneumoniae* to develop resistance to clinically important antimicrobial agents. However, it is important to note that this study focused only on three types of ESBL genes and investigated only the OmpK35 gene. There are other genes, such as AmpC-type genes, and other porins that should be explored to further understand the resistance mechanisms of *Klebsiella pneumoniae*.

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