ASSOCIATION OF BLAOXA-1 GENE WITH MULTIDRUG RESISTANCE IN K. PNEUMONIAE CLINICAL ISOLATES

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ABSTRACT

OBJECTIVES

This study aimed to isolate K. pneumoniae from patients samples and find an association of the plasmid-mediated bla-OXA-1 gene with multidrug-resistant K. pneumoniae.

METHODOLOGY

This cross-sectional study was conducted at Mardan Medical Complex and Khyber Medical University Peshawar. K. pneumoniae was isolated from pus, urine and blood samples by culture and confirmed by biochemical techniques. Antibiotic susceptibility was done by disc diffusion according to the CLSI 2022 guidelines. A polymerase chain reaction was done for the gene after extraction and amplification of plasmid DNA. Furthermore, an association of antibiotic resistance was confirmed with blaOXA-1.

RESULTS

A total of 160 K. pneumoniae isolates were cultured from the patient's samples, including pus (135, 84.37%), urine (15, 9.37%) and blood (10, 6.26%). There were 154 (96.3%) isolates resistant to Penicillin-G, followed by Ceftriaxone 151 (94.4%), Cefepime 143 (89.4%), Amoxicillin 125 (78.1%), Tigecycline 110 (68.8%), Imipenem 92 (57.6%) and Ertapenem 75(49.9%). However, Tetracycline had 1.9% resistance. The blaOXA -1 gene was positive in 41(25.62%) isolates with a different pattern of antibiotics resistance to Penicillin-G, Ceftriaxone, Cefepime, Amoxicillin, Tigecycline, Imipenem and Ertapenem as compared to the negative isolates. Among the blaOXA-1 gene-positive K. pneumoniae isolates, resistance to Penicillin-G was 100%, followed by Ceftriaxone (92.7%), Cefepime and Amoxicillin (80.5%), respectively. However, resistance to Imipenem and Ertapenem was 46.3% and 41.5%, respectively, and Tetracycline was not resistant.

CONCLUSION

Our data suggest that the presence of plasmid associated blaOXA-1 gene in K. pneumoniae isolates may contribute to multidrug resistance in beta lactamase-containing antibiotics along with other internal mechanisms of resistance present in these bacteria.

KEYWORDS: *Klebsiella Pneumoniae, Gene, Antibiotics, Drug Resistance*

INTRODUCTION

Klebsiella Pneumoniae (K. pneumoniae) is a gram-negative, capsule-bearing, non-motile, lactose fermenting bacterium growing in mucoid colonies on MacConkey agar.[1] It is a common causative pathogen of hospital-acquired nosocomial infections, e.g. urinary tract infections, pneumonia, septicaemia and tissue infections.[2,3] They have been grown from inanimate objects like medical devices, and the gastrointestinal tract and healthcare providers hands act as a basic reservoir for K. pneumoniae dissemination.[4] The rapid dissemination of these bacteria in healthcare settings often leads to epidemics.[5] They also cause opportunistic infections such as pneumonia, urinary tract infection, bloodstream infections and sepsis in immune-compromised patients.[6,7] Like other bacteria, K. pneumoniae also resists antimicrobial drugs by intrinsic and acquired mechanisms. The innate genes responsible for such resistance are present in the bacteria genome to protect them from the effect of antimicrobial drugs. However, some genes are acquired through horizontal transfer and reside on the plasmid.[8,9] Over expression of some genes and mutation may lead to antibiotic resistance.[10] An example of intrinsically resistant bacteria is Extended Spectrum β-lactamase (ESBL) producers that offer resistance against β-lactam antibiotics such as Penicillin, Cephalosporin.[11] Strains of K. pneumoniae can protect themselves from antibiotics by their ability to produce ESBL intrinsically.[12,13] However, the emergence of MDR strains may also have some acquired mechanism that needs to be explored. The ESBL-producing genes like TEM, SHV and OXA confer resistance against Ampicillin, Ticarcillin, Piperacillin and the Cephalosporin group of antibiotics. The blaOXA-1 is

located on plasmids and integron segments of the genome in a wide range of gram-negative bacterial species and has been studied to have an association with resistance to Ampicillin and Cephalosporin groups of antibiotics.[14,15] Different genes have been implicated in the resistance against antibiotics in K. pneumoniae. Although the blaOXA-1 gene has been associated with antibiotic resistance in different pathogens including K. pneumoniae, there is scarce literature about plasmid-associated K. pneumoniae in our population. The present study will explore our setup's possible association of the blaOXA-1 gene with multidrug resistance against commonly used antibiotics.

METHODOLOGY

This cross-sectional study was conducted over six months, from January 2022 to June 2022**.** The Advance Study Board of Khyber Medical University Peshawar and Institutional Ethical Committee IPDM, Khyber Medical University Peshawar, approved the study. Patients samples were collected from the pathology laboratory of Mardan Medical Complex, Mardan, and the study was conducted at the Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar. A total of 160 samples were collected through a non-probability convenient sampling technique. Patients having recurrent urinary tract infections, bacteremia, skin and soft tissue infections, and fever of unknown origin were included in the study. However, those on antibiotic therapy and having other chronic conditions were excluded. Samples were primarily processed on MacConkey and Cystine-Lactose-Electrolyte-Deficient (CLED) Agar for 24 hours and identified as mucoid colonies of K. pneumoniae, further confirmed by biochemical tests. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) 2022 through disc diffusion method using Muller Hinton Agar (MHA) against four classes of antimicrobials Penicillin Cephalosporin, Carbapenems, Tetracyclines to find multidrug-resistant (MDR) strains using criteria of the European Center for disease control and prevention ECDC.[16,17] The stored samples were taken out of -80°C, placed in an incubator for 30-40 min, liquefied, and sub-cultured on MacConkey agar for 24 hours using aseptic techniques. According to the manufacturer's instructions, isolated colonies were used for DNA extraction through plasmid DNA extraction Mini Kit (Cat No.D1100, Package: 50T/100T, Solar-bio life sciences). PCR amplification of the blaOXA gene was carried out using the following primers and cycler thermal conditions;

Forward —5'TTTTCTGTTGTTTGGGTTTT'3
Reverse —5'TTTCTTGGCTTTTATGCTTG'3

Thermal cycler temperature:

Temperature	Time
98°C	1 min
95°C	10 sec
54°C	30 sec
72°C	45 sec
72°C	5 min
12°C	∞ (infinity)

RESULTS

Out of 160 participants, the majority were males, 92(57.5%). The female patients were 68(42.5%). The mean age was 34.64 (SD = 6.47) years, K. pneumonia was cultured from different patient samples. Most samples were from pus 135 (84.4%). However, urine and blood were also present, as shown in figure-1.

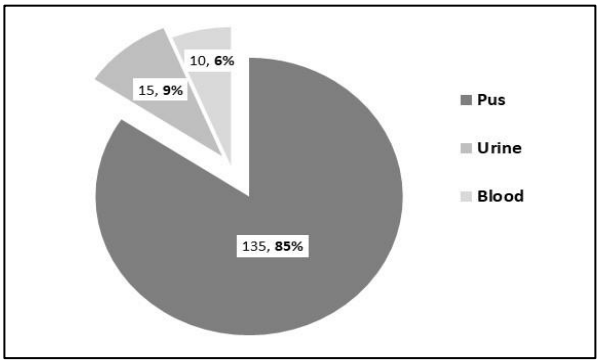

Figure 1: Sample Sources of K. pneumoniae (n=160)

The antibiotic sensitivity pattern of isolates was studied, and the majority, 157 (98.1%) K. pneumoniae, were sensitive to Tetracycline. However, most of the isolates 151 (94.4%) were resistant to Ceftriaxone and other β-lactam antibiotics, as shown in table-1.

Table 1: K. pneumoniae antibiotics sensitivity patterns (n=160)

Antibiotics	Resistance (R)	Intermediate (I)	Sensitive (S)	Total
Tetracycline (TGC)	03 (1.9%)	0(0)	157 (98.1%)	160 (100%)
Ceftriaxone (CRO)	151 (94.4%)	02(1.3%)	07 (4.4%)	
Imipenem (IMI)	92 (57.6%)	25(15.6%)	43 (26.9%)	
Cefepime (FEP)	143 (89.4%)	13(8.1%)	04 (2.5%)	
Ertapenem (ETP)	75 (49.9%)	13(8.1%)	72 (45.0%)	
Penicillin G (P)	154 (96.3%)	0(0%)	06 (3.8%)	
Tigecycline (TE)	110 (68.8%)	07(4.4%)	43 (26.9%)	
Amoxicillin (AMC)	125 (78.1%)	04(2.5%)	31 (19.4%)	

Individual antibiotic susceptibility was assessed, and the highest sensitivity of K. pneumonia was observed to Tetracycline 157 (98.12%), as shown in table-2.

Table 2: Frequency of K. pneumonia Sensitivity to Different Antibiotics

Antibiotics	No. of sensitive samples n (%)	No. of resistant samples n (%)
Tetracycline (TGC)	157(98.12%)	03(1.88%)
Ceftriaxone (CRO)	07(3.13%)	151(96.87%)
Imipenem (IMI)	43(42.5%)	92(57.5%)
Cefepime (FEP)	04(10.63%)	143(89.37%)
Ertapenem (ETP)	72(45.00%)	75(55.00%)
Penicillin (P)	06(3.75%)	154(96.25%)
Tigecycline (TE)	43(31.25%)	110(68.75%)
Amoxicillin(AMC)	31(21.88%)	125(78.12%)

The frequency of the blaOXA-1 gene in K. pneumoniae isolates was assessed, and 41 (25.62%) samples had the blaOXA1 gene out of 160 cases, as shown in Figure 2.

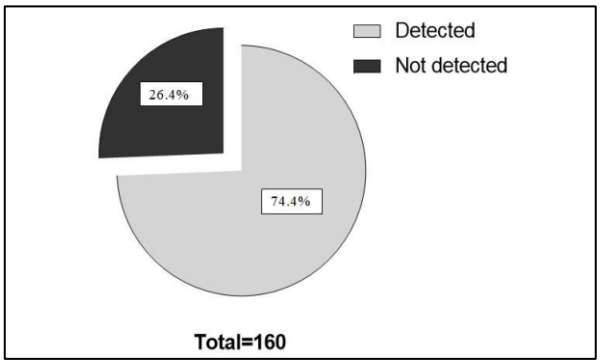

Figure 2: BlaOXA-1 Gene Positivity in K. pneumoniae

Among the blaOXA-1 gene-positive K. pneumonia isolates(41 out of 160), 100% of isolates were resistant to Penicillin G. This was followed by other β lactam antibiotics, including Ceftriaxone (92.7%) and Cefepime and Amoxicillin (80.5%) both. Among β lacatm antibiotics, Imipenem and Ertapenem show resistance at 46.3% and 41.5%, respectively. There was no resistance to Tetracycline, as shown in Table-3.

Table 3: Blaoxa-1 Gene Positive K. pneumonia's Antibiotic Sensitivity

Antibiotics	Resistance (R)	Intermediate (I)	Sensitive (S)	Total
Tetracycline (TGC)	Nil	Nil	41 (100%)	41 (100%)
Ceftriaxone (CRO)	38(92.7%)	Nil	03 (7.3%)	
Imipenem (IMI)	19(46.3%)	09(21.9%)	13 (31.7%)	
Cefepime (FEP)	33(80.5%)	06(14.6%)	02 (4.9%)	
Ertapenem (ETP)	17(41.5%)	01(2.4%)	23 (56.1%)	
Penicillin G (P)	41(100%)	Nil	Nil	
Tigecycline (TE)	24(58.5%)	05(12.2%)	12 (29.3%)	
Amoxicillin (AMC)	33(80.5%)	02(4.9%)	23 (56.1%)	

The overall resistance of isolates of K. pneumoniae having blaOXA-1 gene was different to Ceftriaxone, Amoxicillin, Imipenem, Tegycycline, Penicillin G, Cefepime ($p = 0.001$) as analyzed by Fisher-Exact test. However, Tetracycline did not show a different resistance ($p = 0.40$) analyzed by the Chi-square test, as shown in table-4.

Table 4: Blaoxa-1 Gene Positive K. pneumonia's Antibiotic Sensitivity

	blaOXA-1 gene status	Resistant (R)	Intermediate (I)	Sensitive (S)	Test statistics/ Df	P-Value
Tetracycline (TGE)	Positive	--	0	41	1.05/1	ns 0.40
	Negative	--	03	116		
	*2x2, *Fisher Exact: two cells have an expected count of less than 5					
Ceftriaxone (CRO)	Positive	38	0	03	36.27/6	** 0.001
	Negative	113	02	04		
	*3x2, *Fisher Exact: cells have expected count less than 5					
Cefepime (FEP)	33	06	02		38.15/6	
	110	07	02			
	*3x2, *Fisher Exact: two cells have an expected count of less than 5					
Amoxicillin (AMC)	Positive	33	02	06	34.71/6	
	Negative	92	02	25		
	*3x2, *Fisher Exact: two cells have an expected count of less than 5					
Ertapenem (ERT)	Positive	17	01	23	34.96/6	
	Negative	58	12	49		
	*3x2, *Fisher Exact: one cell has an expected count of less than 5					
Imipenem (IMI)	Positive	19	09	13	28.65/6	
	Negative	71	18	30		
	*3x2, Chi-Square test					
Tigecycline (TE)	Positive	24	05	12	39.43/6	
	Negative	86	02	31		
	*3x2, *Fisher Exact: one cell has an expected count of less than 5					
Penicillin G (P)	Positive	41	0	--	35.24/6	
	Negative	113	06	--		
	*2x2, *Fisher Exact: one cell has an expected count of less than 5					

DISCUSSION

K. pneumoniae has been isolated and cultured from different specimens and can cause infections affecting other body systems. In our hospitals, K. pneumoniae is usually cultured from different patient samples, as shown in our study. 85% of samples were cultured from pus. However, 9% were from urine and 6% from blood in this study. Other studies on K. pneumoniae favour our findings, and researchers have cultured this pathogen from different sources. A survey conducted in 2022 showed that 23% of K. pneumoniae were cultured from pus, 20% from urine and 16% from rectal samples.[26] Similarly, another study showed that respiratory samples had the least number (11%) of K. pneumonia isolates; however, it is the second most common uropathogenic Pakistan.[27] However, another study reported that K.pneumoniae was found in 50% of respiratory samples, followed by pus (16%), blood (11%), urine (19%), stool (59%) and less than 3% from peritoneal fluid and wound swabs. The highest number of K. pneumonia isolates in respiratory samples is explained by the fact that most were collected from the intensive care unit having pneumonia.[28] The K. pneumonia resistance to antibiotics varied by proportion in different studies, but a general pattern of resistance is seen in most of the studies. The pathogen showed higher resistance to the β-lactam antibiotics in general, as shown in our research.[29] In the present study, K. pneumoniae was seen highly resistant to Amoxicillin 125 (78.12%), followed by Tigecycline, 110 (68.75%), Penicillin G 154 (96.25%), Ertapenem 75 (55.00%), Cefepime 143 (89.37%), Imipenem 92 (57.5%), and Ceftriaxone 151 (96.87%) however Tetracycline 3(1.88%) was the most effective which explains the resistance to β-lactam antibiotics. The pattern of resistance seen in our study was also seen in other studies, e.g. beta-lactam antibiotic (Ampicillin (98.6%), cefotaxime (84.7%), cefpirome (81.4%), ceftazidime (79.4%), cefepime (70.8%), cefoxitin (44.3%), piperacillin/tazobactam (39%), ertapenem (24.5%), meropenem (23.8%) and imipenem (22.5%) however these studies showed that other factors like biofilm formation and resistant genes may also contribute to the resistance against these antibiotic.[6,27,29,30] Furthermore, we assessed the association of the blaOXA1 gene with resistance in these isolates. The blaOXA1 gene was detected in 4 out of 160 isolates, and those isolates had statistically strong opposition to the β-lactam mentioned above antibiotics. This reflects the difference in the prescription practices, the trend in the choice of empirical antibiotics and the sites of sample collection of studies. The overall resistance of isolates of K. pneumoniae having blaOXA-1 gene was higher to Ceftriaxone, Amoxicillin, Imipenem, Tegycycline, Penicillin G, Cefepime (p=0.001) as analyzed by Fisher-Exact test. However, Tetracycline did not show any difference in resistance (p=0.40). A similar study showed that blaOXA-1 containing β-lactamase producing strains were highly resistant to piperacillin/tazobactam(100%), levofloxacin (91.6%), amikacin (75%), cefoxitin (50%), ertapenem (25%), imipenem (16.6%) and meropenem (16.6%) which support our finding but all were susceptible to Tigecycline which does not support our results in the present study.[6,27] So, along with the internal resistance of K. pneumoniae, some level of antibiotic resistance is offered by the plasmid-mediated genes, which need to be kept in mind during antibiotic therapy in these infections.

LIMITATIONS

This study focused on one gene and some commonly used antibiotics. Further studies are needed to focus on multiple genes and more antibiotic groups. A whole genome sequencing study is required to find additional mutations.

CONCLUSIONS

The present study concludes that K. pneumoniae isolates from clinical samples exhibit significant resistance to β-lactam antibiotics. Those isolates having the blaOXA-1 gene in their plasmids have significantly higher resistance to beta-lactam antibiotics. However, they may have internal resistance to these antibiotics. These pathogens must be treated with antibiotics other than those having beta-lactam rings in their molecular structure. Among all the eight antibiotics used in this study, K. pneumoniae was highly sensitive to Tetracycline, which can be used as a drug of choice to treat these infections.

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