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DETECTION OF MULTIDRUG RESISTANT *KLEBSIELLA PNEUMONIAE* FROM CLINICAL SPECIMEN COLLECTED FROM GENERAL HOSPITAL, MINNA METROPOLIS, NIGER STATE, NIGERIA

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ABSTRACT
Klebsiella pneumoniae is one of the prominent causes of hospital-acquired and community acquired infections worldwide K. pneumoniae is a common causative agent of various infections and are of great concern due the development of resistance against commonly prescribed antibiotics. This study was carried out to understand the prevalence, identify resistant genes and pattern of spread of multi drug resistant K. pneumoniae isolated from clinical specimens from general hospital, Minna. Standard microbial culturing, gram staining, biochemical and molecular testing were used in this study. The samples were inoculated on MacConkey agar and Eosine Methylene blue agar (EMB). Eighty-eight (88) stools samples, seventy-five urine samples (75) and thirty-seven (37) sputum samples to give a total of two hundred samples were each collected from General hospital Minna, K. pneumoniae had a prevalence of 30.0%. The Kirby Bauer's disc diffusion method was used for the antibiotic susceptibility test, and the results interpreted according to the Clinical and Laboratory Standards Institute guidelines, 2017. *Klebsiella pneumoniae* isolates showed a high resistance to Cephlexin 31(73.8%), ceftriaxone 29(69.0%) and Nalidixic acid 25(59.5%). *K. pneumoniae* was most susceptible to *Trimetoprim*-sulfametoxazole and Streptomycin 39 (92.9%), Ciprofloxacin and Gentamicin 38(90.5%). *K. pneumoniae* showed intermediate resistance to

Amoxicillin clavulanic acid 15(35.7%), Colistin 11(26.2%) and Erythromycin 8 (19.0%). 3 multidrug resistant *K. pneumoniae* isolates were tested for resistant genes; mcr-1, blaTEM blaSHV, FosA, qnrA and qnrB, all 3 (U1, U2, ST6) isolates were positive for mcr-1 which is associated with colistin resistance, U1 and ST6 were positive for blaTEM, U2 was positive for the presence of blaSHV, FosA and qnrB genes, all isolates were negative for qnrA. The study confirmed there's a high prevalence MDR *K. pneumoniae* in Minna, Nigeria. Routine surveillance and more research into the pattern of spread and resistant genes is recommended.

Keywords: Klebsiella pneumoniae, Antibiotic susceptibility, Antimicrobial resistance, Multi drug resistant, Minna

Introduction

Klebsiella pneumoniae is one of the prominent causes of hospital-acquired and community acquired infections worldwide (Kashefieh *et al.,* 2021). K. pneumoniae is a common causative agent of different infections and are of great concern due the development of resistance against commonly prescribed antibiotics (Sonia *et al.,* 2020). This pathogen **frequently causes lower respiratory tract infections, Bacteraemia, Urinary tract infections (UTI) and wound infections (**Kurhade *et al.,* 2015**).** It **is the second most common cause (behind** Escherichia coli**) of community and hospital-acquired gram-negative bloodstream infection (**Meatherall *et al.,* 2009**). Antimicrobial resistance among clinical isolates of** K. pneumoniae **has become an increasingly serious problem over the past twenty years (**Calfee, 2017**). Multidrug resistant strain of** K. pneumoniae **emerged due to indiscriminate use of various antibiotics (**Du *et al.,* 2014**).**

Klebsiella pneumoniae is one of the three major drug-resistant bacteria on the WHO priority list that requires more research and production of novel antibiotics for their treatment (Caneiras *et al.,* 2019). Multi drug resistance (MDR) against different classes of antibiotics had made this pathogen become a serious threat in health-care facilities, which in turn limits

treatment options of infections they cause (Navon-Venezia *et al.,* 2017). This pathogen uses various resistance mechanisms including production of antibiotic hydrolysing enzymes, efflux pumps, porin loss, and target alteration to counteract the effects of antibiotics (Ferreira *et al.,* 2019). *K. pneumoniae* like other opportunistic pathogens affect patients who have predisposing debilitating backgrounds and are mainly reported from ICUs, urinary tract infections, ventilator-associated pneumonia, and sepsis (Xu *et al.,* 2019).

Aim and Objectives of The Study

The aim of the study is to detect multidrug resistant *Klebsiella pneumoiae* in clinical samples collected from General Hospital Minna, Niger state, Nigeria

The Objectives of the study are to:

i. isolate and identify *Klebsiella pneumoniae* from clinical specimen.
ii. determine the antibiotic susceptibility profile of *Klebsiella pneumoniae*
iii. identify genes encoding for antibiotic resistance using molecular techniques

Materials and method

A total of 200 clinical specimen were collected, these specimens were stool (88), urine (75) and sputum (37), samples were collected in a sterile sample bottle. Standard microbial culturing, gram staining, biochemical and molecular testing were used in this study. The Kirby Bauer's disc diffusion method, antibiotic susceptibility test was performed with the results interpreted according to the Clinical and Laboratory Standards Institute guidelines, 2017, this was used to determine the susceptibility patterns of *K. pneumoniae* isolates. *Klebsiella* isolates identification was carried out using the 16S rRNA sequence analysis. 3 of the isolates were investigated for the presence of blaSHV, blaTEM, FosA, qnrA, qnrB, and mcr-1 via polymerase chain reaction (PCR) using gene specific primers

Results

A total of 200 clinical samples were collected and isolated on MacConkey agar and Eosine methylene blue (EMB) agar. The clinical samples were urine, sputum, and stool; of the 200 samples, 122 showed positive growth on MacConkey agar and Eosine methylene blue (EMB) agar while 78 plates were negative with no growth.

Molecular Identification of Isolates

Klebsiella pneumoniae isolates identification was carried out using the 16S rRNA sequence analysis. Plate 1 shows the agarose results of 16S rRNA PCR amplified products from extracted DNA of *K. pneumoniae* isolates. All 3 suspected isolates of *K. pneumoniae* were confirmed as shown in the gel image with horizontal lines across all lanes. The electrophoresis was performed for 90 minutes at 70 volts.

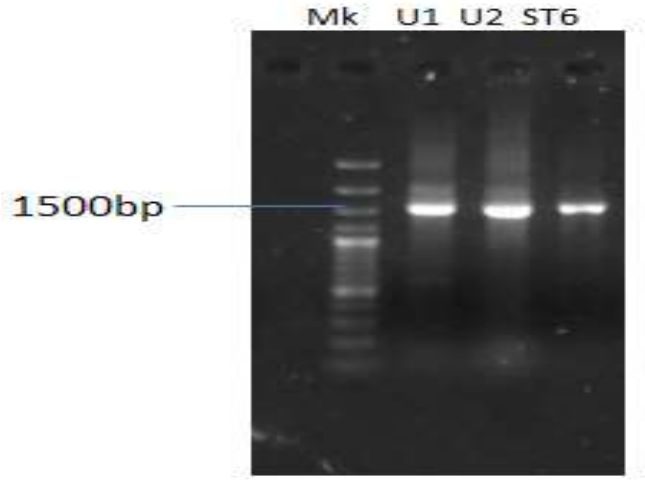

Plate 1 Agarose gel electrophoresis of the PCR products of the 16S rRNA gene amplified from three bacteria isolates. (Band size approximately 1500bp).

Antimicrobial susceptibility profile of *Klebsiella pneumoniae* isolates

The Kirby Bauer's disc diffusion method was used to perform the antibiotic susceptibility test the results interpreted according to the Clinical and Laboratory Standards Institute guidelines, 2017. Table 1 below shows the antimicrobial susceptibility profile of *Klebsiella pneumoniae*

Table 1: Antimicrobial susceptibility profile of *Klebsiella pneumoniae* isolates

S/N	Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
1	CPX	38 (90.5)	3 (7.1)	1 (2.4)
2	CN	38 (90.5)	3 (7.1)	1 (2.4)
3	CEP	7 (16.7)	4 (9.5)	31 (73.8)
4	NA	13 (31.0)	4 (9.5)	25 (59.5)
5	AMC	5 (11.9)	15 (35.7)	22 (52.4)
6	E	29 (69.0)	8 (19.0)	5 (11.9)
7	FOS	31 (73.8)	5 (11.9)	6 (14.3)
8	CT	25 (59.5)	11 (26.2)	6 (14.3)
9	CH	31 (73.8)	7 (16.7)	4 (9.5)
10	TET	31 (73.8)	4 (9.5)	7 (16.7)
11	SXT	39 (92.9)	2 (4.8)	1 (2.4)
12	PN	13 (31.0)	5 (11.9)	24 (57.1)
13	CET	7 (16.7)	6 (14.3)	29 (69.0)
14	S	39 (92.9)	1 (2.4)	2 (4.8)
15	AMX	22 (52.4)	8 (19.0)	12 (28.6)

CPX: Ciprofloxacin; NA: Nalidixic acid; PN: Ampicillin; AMC: Amoxicillin clavulanic acid; CEP: Cephalexin; CN: Gentamicin; S: Streptomycin; CH: Chloramphenicol; SXT: Trimetoprim-sulfametoxazole; FOS: Fosfomycin; CT: Colistin; TET: Tetracycline; CET: Ceftrixone; E: Erythromycin; AMX: Amoxicillin.

Multiple Antibiotic Resistance Index (MARI)

The Multiple Antibiotic Resistance index (MARI) was also determined by following the procedure described by Krumperman (1983). The formula used to calculate the MAR index for each isolate is given below as equation 1.1.

$$\text{MAR index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics against which isolate was tested}} \quad (1.1)$$

Table 2: Multiple Antibiotic Resistance Index (MARI)

MARI	Number of isolates	Percentage %
0	1	2.4
0.1	3	7.1
0.2	10	23.8
0.3	19	45.2
0.4	5	11.9
0.5	4	9.5
0.6	0	0.0
Total	**22**	**100**

The isolates were categorized into susceptible, Non MDR, and MDR, of the 42 *Klebsiella* isolates, 61.9% was Non Multi drug resistant (MDR), 35.7% were Multi drug resistant (MDR) and 2.4% was susceptible. Table 3, shows the resistance category of *Klebsiella* isolates,

Table 3. Resistance category of *Klebsiella* isolates,

Resistance category	Number of isolates	Percentage %
Susceptible	1	2.4
Non MDR	26	61.9
MDR	15	35.7

Identification of Resistance Genes

The gel electrophoresis plates of PCR performed using extracted DNA from isolates U1, U2, ST6 indicated the presence of TEM, SHV, qnrA, qnrB, FosA, mcr-1 with 320bp, 271bp, 258bp, 320bp and 250bp amplicon size (Plate 2,3,4,5). Table 4 shows the antibiotics resistant genes of *K. pneumoniae*

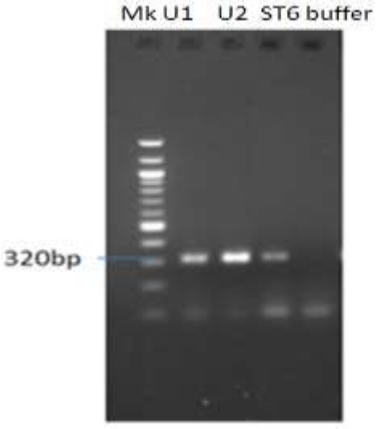

Plate 2 Agarose gel electrophoresis of the PCR products of Mcr-1 gene amplified from three bacteria isolates identified as *Klebsiella pneumoniae*. (Band size approximately 320bp). Gel image indicates a positive amplification in all samples indicating the presence of Mcr-1 gene

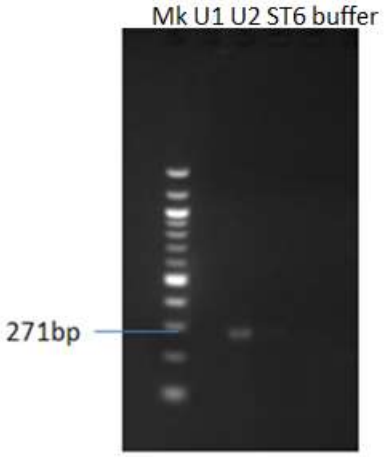

Plate 3 Agarose gel electrophoresis of the PCR products of fosA gene amplified from three bacteria isolates identified as *K. pneumoniae*. (Band size approximately 271bp). Gel image indicates a positive amplification in only U2 isolate.

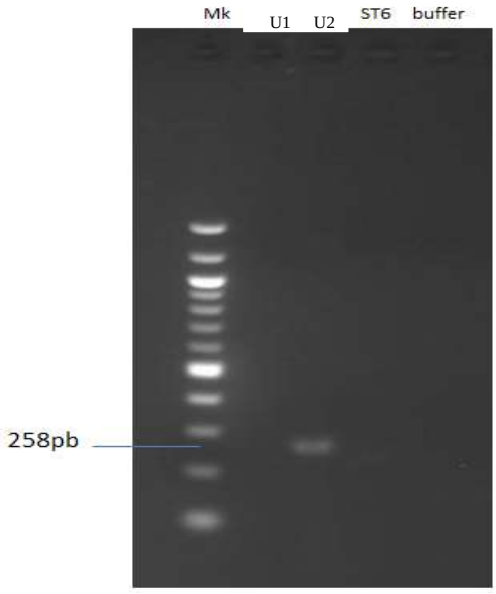

Plate 4 Agarose gel electrophoresis of the multiplex PCR products of qnrA and qnrB gene amplified from three bacteria isolates identified as

Klebsiella pneumoniae. (Band size approximately 258pb for qnrB and 580bp for qnrA). Gel image indicates a positive qnrB amplification in only U2 isolate while all were negative to qnrA.

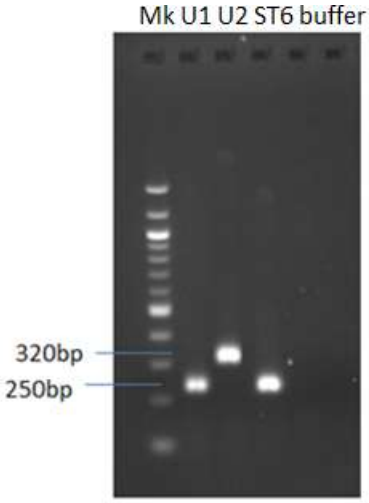

Plate 5 Agarose gel electrophoresis of the multiplex PCR products of SHV and TEM gene amplified from three bacteria isolates identified as *Klebsiella pneumoniae*. (Band size approximately 258pb for blaTem and 320bp for blaSHV). Gel image indicates a positive SHV amplification in only U2 isolate and a positive TEM amplification in U1 and ST6.

Table 4. Antibiotic Resistant genes of *Klebsiella pneumoniae*

Isolate Code	SHV	TEM	FosA	qnrA	qnrB	Mcr 1
U1	-	+	-	-	-	+
U2	+	-	+	-	+	+
ST6	-	+	-	-	-	+

Keys: +: Present; -: Absent.

Discussion

Klebsiella pneumoniae is a common pathogen associated with both community and hospital-acquired infections including respiratory, urinary tract, wound and blood infections Lagha *et al*., 2020. In this study, 42 *K. pneumoniae* were isolated from urine, stool, and sputum of patients. The distribution of K. pneumoniae was slightly higher for male patients.

This study showed that *Klebsiella pneumoniae* has a prevalence of 30% this finding is like the study carried out by Farkhanda *et al* 2019, Oyedum *et al.,* 2022 and Kaapul *et al.,* 2022 with a prevalence of 36.5%, 21.3% and 23%. Samples from the age group of 16-25 had the highest *K. pneumoniae* occurrence of 33.3% while the age group ≥76 had the least occurrence.
Of the 200 clinical samples collected, 106 (53.0%) were collected from males and 94 (47.0%) were collected from females, *K. pneumoniae* had a prevalence of 23 (54.8%) in the 106 samples collected from males while 19 (45.2%) was present in the samples collected from the females
Forty-two *K. pneumoniae* were obtained in this study, of the 42 isolates 26 were non-Multidrug resistant which account for 61.9% while 15 were multidrug resistant which accounted for 35.7%, this finding agrees with studies carried out by Kaapul *et al.,* 2022 with a prevalence of MDR *Klebsiella pneumoniae* of 32.4%.
A total of 15 antibiotics was used for the antibiotic sensitivity test, *Klebsiella pneumoniae* isolates showed a high resistance to Cephlexin 31(73.8%), ceftriaxone 29(69.0%) and Nalidixic acid 25(59.5%). *K. pneumoniae* was most susceptible to *Trimetoprim*-sulfametoxazole and Streptomycin 39 (92.9%), Ciprofloxacin and Gentamicin 38(90.5%). *K. pneumoniae* showed intermediate resistance to Amoxicillin clavulanic acid 15(35.7%), Colistin 11(26.2%) and Erythromycin 8 (19.0%)..
The high susceptibility to *Trimetoprim*-sulfametoxazole and streptomycin, Ciprofloxacin and Gentamicin from this study is in disagreement with findings from Oyedum *et al.,* 2022 and Kaapul *et al.,* 2022, while Susceptibility to Ciprofloxacin (90.5%) is in line with a study by Akinyemi *et al,* 2018 and Kaapul *et al.* 2022 with *Klebsiella pneumoniae* having a susceptibility of 72.1%, and 69.6%, while Gentamicin (90.5%) when compared with a study by Kaapul *et al.* 2022 had a susceptibility of 48.4%.
Significant high resistance of *K. pneumoniae to* Cephlexin (73.8%) and Ceftriaxone (69.0%), may be due to the production of β-lactamase enzymes which cause the hydrolysis of the β-lactam ring resulting in inactivation of β-lactam antibiotics such as 1st and 3rd generation cephalosporins. The β-lactam antibiotics dose, as well as the incidence of toxicity, subsequently reduced if beta-lactamase inhibitors are used it.

Another mechanism is associated with penicillin-binding proteins, this agrees with findings of Farkhanda *et al* 2019.

3 multidrug resistant *K. pneumoniae* (U1, U2, ST6) isolates were tested for resistant genes; mcr-1, blaTEM blaSHV, FosA, qnrA and qnrB, all 3 isolates were positive for mcr-1 which is associated with colistin resistance, U1 and ST6 were positive for blaTEM, this could be associated to their resistance to Cephalexin, Nalidixic acid, Amoxicillin clavulanic acid, Colistin, Ceftrixone, Amoxicillin, Chloramphenicol and Ampicillin while, U2 was positive for the presence of blaSHV, FosA and qnrB genes this could be associated with its resistance to Cephalexin, Nalidixic acid, Amoxicillin clavulanic acid, Fosfomycin, and Ceftrixone all isolates were negative for qnrA.

Other factors for *K. pneumoniae* virulence includes capsules, exopolysaccharides associated with mucoviscosity, lipolpolysaccharides (LPS), adhesins and iron uptake systems. This fsctors aggravate the infection caused by *K. pneumoniae.*

Conclusion/Recommendations

Conclusion

The high antibiotic resistance of *Klebsiella pneumoniae* towards commonly prescribed antibiotics are the most profound reasons for prolonged infections, longer duration of hospitalization, high cost of therapy and increased morbidity and mortality rates.

Klebsiella pneumoniae was found to be prevalent amongst the age group 0f 16-25 and higher in male. It is found to be most susceptible to *Trimetoprim*-sulfametoxazole and streptomycin, Ciprofloxacin, and Gentamicin.

Recommendations

Based on the findings of this research, the following recommendations are made

1. Strict measure to be placed in the prescription and dispensation of antibiotics.
2. Regular surveillance of antibiotic susceptibility patterns may help to overcome the rash use of antibiotics which has been found to be a major cause of the emergence of MDR among *Klebsiella pneumoniae.*

3. More research to be conducted on the pattern of spread and genes that encodes for resistance to antibiotics
4. There is a need for public health workers, to create awareness on the misuse of antibiotics and promote effective use of antibiotics, to prevent and minimize treatment failure due to antibiotic resistance

References

Calfee, D. P. (2017). Recent advances in the understanding and management of Klebsiella pneumoniae. F1000Research, 6.

Caneiras, C., Lito, L., Melo-Cristino, J., & Duarte, A. (2019). Community-and hospital-acquired *Klebsiella pneumonia*e urinary tract infections in Portugal*: virulence and antibiotic resistance. Microorganisms, 7(5), 138.*

Du, J., Li, P., Liu, H., Lü, D., Liang, H., & Dou, Y. (2014). Phenotypic and molecular characterization of multidrug resistant *Klebsiella pneumoniae* isolated from a university teaching hospital, China. *PloS one, 9(4), e95181*.

Ferreira, R. L., da Silva, B., Rezende, G. S., Nakamura-Silva, R., Pitondo-Silva, A., Campanini, E. B., & Pranchevicius, M. C. D. S. (2019). High prevalence of multidrug-resistant *Klebsiella pneumoniae* harbouring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. *Frontiers in microbiology, 9, 3198.*

Kaapu, K.G., Maguga-Phasha, N.T., Seloma, N.M., Nkambule, M.C. & Lekalakala-Mokaba, M.R. (2022). Prevalence and Antibiotic Profile of Multidrug Resistance Gram-Negative Pathogens Isolated from Wound Infections at Two Tertiary Hospitals in Limpopo Province, South Africa: *A Retrospective Study. Open Journal of Medical Microbiology, 12, 141-155* https://www.scirp.org/journal/ojmmx

Kashefieh, M., Hosainzadegan, H., Baghbanijavid, S. & Ghotaslou, R. (2021). The Molecular Epidemiology of Resistance to Antibiotics among *Klebsiella pneumoniae* Isolates in Azerbaijan, Iran. *Journal of Tropical Medicine*.

Kurhade, A., Akulwar, S., Mishra, M., Kurhade, G., Justiz-Vaillant, A., Kurhade, K. & Lakhdive, S. (2015). Bacteriological study of post-operative wound infections in a tertiary care hospital. *Journal of Bacteriology & Parasitology, 6(6), 1.* https://doi.org/10.4172/2155-9597.1000251

Lagha, R., Abdallaha, F.B., ALKhammasha, A.A.H, Amor, N., Hassan, M.M., Mabrouka, I., Alhomrani, M. & Gaber, A. (2021). Molecular characterization of multidrug resistant Klebsiella pneumoniae clinical isolates recovered from King Abdulaziz Specialist Hospital at Taif City, Saudi Arabia. *Journal of Infection and Public Health* 14, 143–151. http://www.elsevier.com/locate/jiph

Meatherall, B. L., Gregson, D., Ross, T., Pitout, J. D., & Laupland, K. B. (2009). Incidence, risk factors, and outcomes of Klebsiella pneumoniae bacteremia. The American journal of medicine, 122(9), 866-873.

Navon-Venezia, S., Kondratyeva, K., & Carattoli, A. (2017). Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS microbiology reviews, 41(3), 252-275.*

Oyedum, M.U., Kuta, F.A., Saidu, A.N. & Babayi H. (2022). Isolation of multidrug-resistant Escherichia coli and Klebsiella pneumoniae from urogenital samples of patients with Pelvic inflammatory disease in North Central Nigeria. *Biomed Natural and Applied Science 2(1),37-45* https://doi.org/10.53858/bnas020103745 ISSN: 2789-178

Sonia, S. J., Afroz, S., Rasheduzzaman, M., Uddin, K. H. & Shamsuzzaman, S. M. (2020). Prevalence and antimicrobial susceptibility pattern of Klebsiella Pneumoniae isolated from various clinical specimens in a tertiary care hospital in Bangladesh. *Medicine Today*, 32(2), 95-99.

Xu, H., Huo, C., Sun, Y., Zhou, Y., Xiong, Y., Zhao, Z., & Chen, Y. (2019). Emergence and molecular characterization of multidrug-resistant Klebsiella pneumoniae isolates harboring blaCTX-M-15 extended-spectrum β-lactamases causing ventilator-associated pneumonia in China. *Infection and drug resistance, 12, 33.*