OPEN ACCESS
Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Molecular Detection of MCR-1 Gene Colistin Resistance in *Klebsiella pneumoniae* Isolated from Clinical Samples in Khartoum State Hospitals, Sudan 2021

Mohamed Qaje Adan[1,2], Mahadi Hassan Mahmoud[1], Ismail Adam Arbab[2,3*], Aisha A. Samatar[2]

[1]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Medical Sciences & Technology, Khartoum, Sudan
[2]Department of Medical Laboratory Sciences, Faculty of Medicine and Health Sciences, University of Bosaso, Garowe Campus, Puntland State, Somalia
[3]Associate Professor, Department of Chemistry, Faculty of Education, West Kordufan University, West Kordufan State, El-Nuhud 55511, Sudan

DOI: 10.36348/sjbr.2022.v07i12.004 | **Received:** 25.10.2022 | **Accepted:** 06.12.2022 | **Published:** 14.12.2022

***Corresponding author:** Dr. Ismail Adam Arbab
Department of Medical Laboratory Sciences, Faulty of Medicine and Health Sciences, University of Bosaso, Garowe Campus, Puntland State, Somalia

Abstract

Background: Antimicrobial Resistance is a major concern because a resistant infection may kill, and can spread to others, and imposes huge costs to individuals and society. The prevalence of MCR-1 resistance gene in Sudan possesses potential threat for treatment of patient especially when hospitalized. This study was implemented to determine the prevalence of MCR-1 Resistance gene in Khartoum state Hospitals in *K. pneumoniae*, isolates from clinical specimens using Polymerase Chain Reaction (PCR) technique. ***Methods:*** In this study, one hundred *Klebsiella pneumoniae* isolates from clinical specimens the study was (31%) were males, while (69 %) were females. There were aged from (17 to 40) years with (Mean = 27.4 and SD = 6.598). The isolated organisms were stored in 20% Glycerol Peptone Media. Samples were cultured and Re-identified using conventional methods. And tested to antimicrobial susceptibility test using Kirby baur disk diffusion Method. ***Results:*** 11% were Resistance for AST to colistin. then presence of MCR-1 gene was investigated by Polymerase Chain Reaction (PCR) technique. Out of 100 of *Klebsiella pneumoniae* Isolates, 4(4%) show positive MCR-1 Three of them from these 11% Resistance and only one from other 89 sensitive the data computerized and analyzed through SPSS 23. All statistical tests have done using chi-Square and the association of Colistin Resistance with MCR-1 gene were significant p-value (<0.05). The Colistin Resistance is an alarm from staff holders because it is considered as main treatment for ICU infections.

Keywords: MCR-1 gen, *Klebsiella pneumoniae*, antimicrobial susceptibility test, polymerase chain reaction.

1. INTRODUCTION

The antimicrobial resistance occurs when microorganisms such as bacteria, viruses, fungi, and parasites change in ways that render the medications used to cure the infections they cause ineffective. When the microorganisms become resistant to most antimicrobials they are often referred to as "superbugs. This is a major concern because a resistant infection may kill, can spread to others, and imposes huge costs to individuals and society [1]. The Multidrug resistance in Gram-negative bacteria, specially Enterobacteriaceae like *E. coli* and *K. pneumoniae* Enterobacter, and Citrobacter has become one of the most important global problems. The Infections of that Enterobacteriaceae cause prolonged hospital admissions and higher mortality rates [2].

Internationally there is a growing concern over antimicrobial resistance (AMR) which is currently estimated to account for more than 700,000 deaths per year worldwide. If there's not appropriate measures are taken to halt its progress, AMR will cost approximately 10 million lives and about 100$ trillion per year by 2050 In contrast to some other health issues, AMR is a problem that concerns every country irrespective of its level of income and development as resistant pathogens

do not respect borders despite the threat presented by AMR, the 2014 World Health Organization (WHO) and the recent O'Neill report describe significant gaps surveillance, standard methodologies and data sharing . The 2014 WHO report identified Africa and South East Asia as the regions without established AMR surveillance systems. This lack of quality data is problematic often leading to treatment guidelines that are not adequate for the local [3].

Klebsiella pneumoniae, which belongs to the Enterobacteriaceae, it is a bacterial pathogen commonly related to hospital infections or hospital acquired infection, including pneumonia, bloodstream infection, Urinary tract infection (UTI), and liver abscess, especially among immunocompromised patients. Carbapenem has been used because the drug of choice for treating infections caused by multidrug-resistant, *Klebsiella pneumoniae*, However, the increased prevalence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), significantly impaired the efficacy of carbapenem antibiotics. then they prefer to treat Colistin drug [4]. *Klebsiella pneumoniae* is an opportunistic bacterial pathogen known for its high frequency and diversity of antimicrobial resistance (AMR) genes. In addition to being a significant clinical problem, K. pneumoniae is the species within which several new AMR genes were first discovered before spreading to other pathogens (e.g., carbapenem-resistance genes KPC, OXA-48 and NDM-1). Whilst *K. pneumoniae's* contribution to the overall AMR crisis is impossible to quantify, current evidence suggests it has a wider ecological distribution, significantly more varied DNA composition, greater AMR gene diversity and a higher plasmid burden than other Gram-negative opportunists. Hence, we propose it plays a key role in disseminating AMR genes from environmental microbes to clinically important pathogens [5].

2. MATERIALS & METHODS

2.1. Study design, study area and duration:

This study was a cross-sectional hospitals-based study. This study was conducted in Khartoum State hospitals. *K. pneumoniae* isolates were collected from the following hospitals; Royal Care International Hospital, Al-Baraha Hospital, Soba University Hospital, East Nile Model Hospital, Omdurman Medical Military Hospital. The study was performed at University of Medical Science and Technology and AL-Neelain University, during the period from April to Juley, 2021.

2.2. Study population & ethical consideration:

The sample population were unknown but isolates were Represent the sample population. All information we collect will be confidential and will be shared with patient and Personal information will not be published and we will publish the results to publish interest and the right of refusal or withdrawal is guaranteed to all participants in this study at any stage. The patient will be provided with full information and procedures for this study, the patient's consent will be taken after knowing the full information.

2.3. Data collections & sample size:

Data were collected from hospital records. A total of 100 identified *K. pneumonia* isolates from various clinical specimens: swab, blood and urine and sputum. $\boldsymbol{n = z\ 2p\ q\ d2}$ = 3.84 × 0.5×0.5/ 0.0025 = 384 Since the operating costs of using PCR are very expensive and the situations that Sudan is going through,100 samples were included in this study.

2.4. Laboratory Method:

2.4.1. Pathogen, nutrient Agar & staining:

Basic medium was used for purification and support the growth of bacteria. A bacterial colony was suspended in normal saline, smeared, left to dry, fixed with heat by passing slide three times over flamed heat and then stained with crystal violet stain for 30–60 seconds, Washed, covered with Lugol's iodine for 30–60 seconds, decolorized rapidly (few seconds) with acetone–alcohol and washed and finally covered with safranin stain for 2 minutes, washed and dried. The dried smear examined microscopically by oil immersion.

2.4.2. Biochemical Identification:

The tests were carried out on Kligler iron agar (KIA), tryptophan peptone water, Simmons's citrate agar and Christensen's urea agar and results was reported. All tests have been done according to standard laboratory procedures [14].

2.4.2.1. Kligler Iron agar:

Kligler Iron Agar medium was prepared according to the instructions of manufacturer. The sterilized straight wire was used for inoculation the butt was stabbed and then the slope streaked in a zig-zag pattern. Observation for color changes, gas and H_2S production was done after 24 hours of incubation at 37^{o}C. A yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose, this occurs in bacteria such as *E. coli* and *K. pneumoniae*. A yellow butt (acid production) and red-pink slope indicate the fermentation of glucose only, as in Proteus 14 Spp and *Pseudomonas spp.* gas production from glucose fermentation was observed as cracks and bubbles in the medium, this was showed in *K. pneumoniae* [19].

2.4.2.2. Indole test:

Tryptophan peptone water was used. The test organism was inoculated in a bijou bottle containing about 3 ml of sterile tryptone water, and sealed well then test tube was incubated under a septic condition at 37˚C for up to 48h. Production of indole was checked by addition of 0.5 ml of Kovac's reagent, examined for a red color in the surface layer within 10 minutes after shaked gently which indicate positive result. Positive result was observed in most strains of *E. coli*, *P.*

vulgaris, P. rettgeri, M. morganii, and Providencia species. Other Enterobacteriaceae are indole negative (no color change) [20].

2.4.2.3. Citrate test:

The tested organisms were suspended in a normal saline and cultured in a Simmons's citrate agar (HIMEDIA, India) By using a sterile straight wire, firstly the slope was streaked and then butt was stabbed and incubated over night at 37^{o}C. This test was used to assist in differentiation of E*nterobacteriaceae spp., K. pneumonia* and some strains of *Proteus Spp* give positive result (bright blue). *E. coli* is citrate negative (no color changes) [19].

2.4.2.4. Urease test:

A medium which contains urea and phenol red as indicator was used for test organism culture in by using a sterile straight wire and under a septic condition, the media was inoculated and incubated overnight at 37^{o}C. This test was used to differentiate *Enterobacteriaceae spp.* positive result observed as production of pink color as seen in Proteus strains (strong Urease producers), *K. pneumoniae* (slow) and *E. coli* is Urease negative [20].

2.4.2.5. Antimicrobial susceptibility test:

All identified isolates were subjected to antibiotic susceptibility testing discs by Kirby-Bauer diffusion method by Muller-Hinton agar was prepared according to lab direction. Suspension from tested organism was prepared by emulsified few colonies in sterile normal saline and compared with turbidity of 0.5 McFarland standard. Cotton swab was used for inoculate suspension on Muller-Hinton agar surface after dipped into standardized suspension and squeezed genteelly to remove excess from swab. Under aseptic condition and by using sterile 15 forceps the following antimicrobial disc were applied on the surface of the agar Cefoxitin (30µg), meropenem(10µg), Amikacin (10µg), Cefotaxime (30µg), Imipenem (10µg) and Colistin (10µg), the distance performed was 24mm between discs and 10 mm between discs and plate edges. The plate was incubated aerobically in incubator at 37°C for overnight. The plate was examined and ruler was used to measure zone diameter in (mm). The recorded zones were compared with those in the chart; results with specific organism reported as resistant (R) or sensitive (S) [14].

2.4.3. Detection of MCR-1 by Polymerase chain reaction

2.4.3.1. DNA extraction Boiling method was used for extracting DNA

The (3-5) colonies from tested organisms were dissolved in 1ml of sterile distilled water, boiled for 10 minutes in a water bath, and then were centrifuged for 5minutes at 1000 rpm.

2.4.3.2. Primer:

Primers Sequences used for the Amplification of Gene are Primer Name DNA sequence (5' to 3') Amplicons size (bp) MCR-1 Forward: (5'CGGTCAGTCCGTTTGTTC-3') Reverse: (5'-CTTGGTCGGTCTGTAGGG-3) 309 was used for screening as previously described. Stock primer Two hundred fifty µl of sterile DW was added to each vial of primer then vortex was performed. Working Primer Ninety µl of distilled water was added to (10 µl) of stoke primer for dissolving and stored at (- 20 ºC). Master Mix Maxime PCR PreMix kit, (iNtRON Biotecnology, seongnam korea). Reaction Mixture PCR was carried out in a 15 µl volume using the Maxime PCR PreMix, (i-Taq) which used for detection of MCR-1 gene in the following volumes We added 5µl of master mix + 5µl of double distilled water + .5 µl of each primer (forward and reverse), and 4 µl of extracted DNA as template.

Protocol used for amplification of the MCR-1gene The amplification was done by using thermocycler PCR (TECHNE) The PCR mixture was subjected to initial denaturation step at 94°C for 5-min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 51°C for 1 min, followed by step of extension at 72°C for 1 min and the final elongation at 72°C for 10 min.

2.4.4. Gel Electrophoresis

2.4.4.1. Preparation of Agarose Gel:

Amount of 1.5 g of agarose powder (BIOLINE, UK) was dissolved in (100 ml) 1X TBE buffer (Sigma, Japan) in microwave for 2 min for heating. The mixture was cooled, 2 µl of (20mg/ml) ethidium bromide was added. Taped up the casting tray appropriately and equipped with suitable comb to form well in place then split the mixuer on. Bubbles were removed and then gel were setting at room temp to solidify. All casting tray equipment were removed gently.

2.4.4.2. Electrophoresis and Visualization of PCR product:

The 1x TBE buffer (Sigma, Japan) was use to flooded gel casting tray until gel surface was covered, 2µl of 100bp DNA ladder (marker) (INtRON) ladder was added to the first well of casting tray and for each run, then 4 µl of PCR products of each sample were put into each well. The gel electrophoresis apparatus was run at 100 v for 15 min (CONSORT E865, Belgium). The gel was removed and visualized under the ultraviolet Transilluminator. Preparation of 10X TBE buffer Amount of 108 grams of Tris base was added to 55g of boric acid and 40 ml of 0.5 EDTA, and then dissolved into 1 liter Deionized water pH 8.0. Preparation of 1X TBE buffer Ten ml of 1X were added to 90 ml deionized water and stirring until completely dissolved.

2.5 Data analysis:

Data of research was recorded and analyzed by SPSS statistical analysis software version 23.0. Using Chi-Square test for analysis and estimate p value.

3. RESULTS

A total of 100 Sudanese subjects were enrolled in this study to isolates of *k. pneumonia* from different hospitals in Khartoum state and confirmed by fermented lactose and produced smooth mucoid pink colonies then identified by biochemical tests. The ages of patients were ranged from (17- 40) years (Mean ± 27.4 .SD 6.598) as show in **(**Figure 3.2). and (31%) were male and (69%) were female as show in (Figure 3.1). All samples done for antimicrobial susceptibility test (AST) by using Kirby Bauer disc diffusion technique, the result show (89%) was sensitive to Colistin and (11%) were resistance as show in (Table 1).

3.1. Gender

One hundred isolates of *K. pneumoniae* were collected from different hospitals in Khartoum State. (31%) were males and (69%) were females as show in (Figure 3.1).

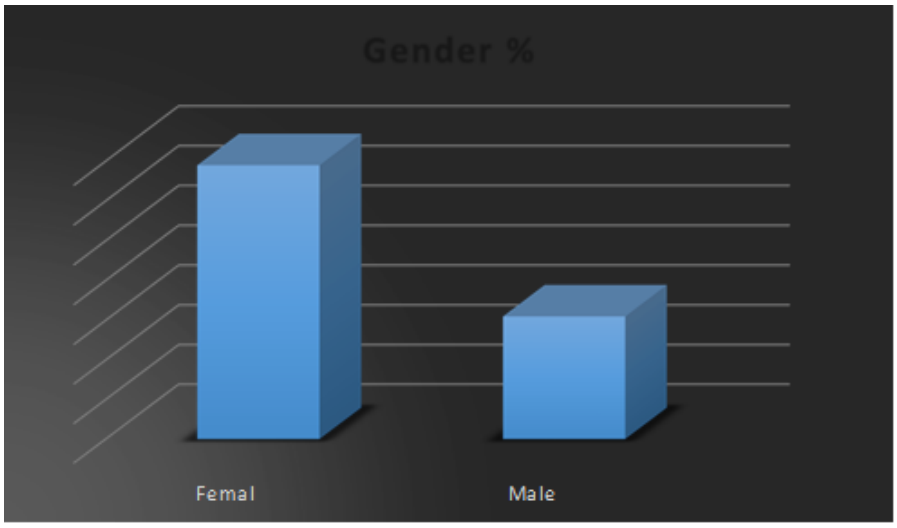

Figure 3.1: Distribution of *K. pneumoniae* in Colistin according to gender

3.2. Detection of K. pneumoniae among study population according to Age

A hundred isolates of *K. pneumoniae* were collected from different hospitals in Khartoum State. (8 %) were <18 years, (78 %) were 19-35 years and (14 %) were >36 years.

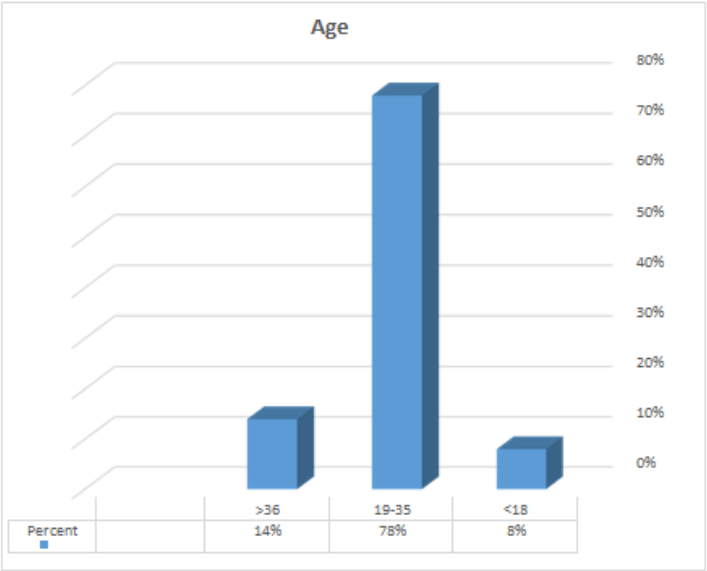

Figure 3.2 Distribution of patients according to age (Mean ± 27.4, SD = 6.598)

3.3. Antibiotics susceptibility test

Out of 100 Isolated samples, (89%) were sensitive to Colistin and (11%). Were resistance by AST show in the table below.

Table 1: Antimicrobial susceptibility test among patients

Antibiotic susceptibility test to colistin			
		Frequency	**Percent**
Colistin	**Resistance**	**11**	11.%
	Sensitive	**89**	89%
	Total	100	100.0

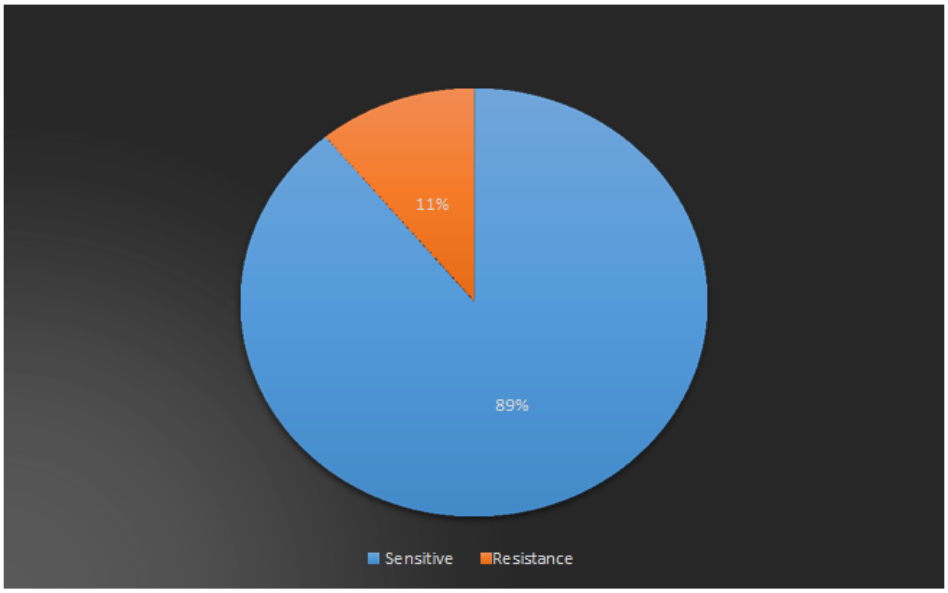

Figure 3.3: Distribution of patients according to sensitive and resistance of Colistin

3.4. Identification of *K. pneumoniae* using PCR method.

All 100 isolates of *K. pneumoniae* identified by using PCR.

Detection of *K. pneumoniae in MCR-1* resistance gene

All 100 *K. pneumoniae* isolates investigated for the presence of Colistin resistance gene *(MCR-1)* using multiplex PCR. There were (4%) positive for *MCR-1* gene and (96%) were negative as show in (figure 3.5.),

Table 2: Frequency of *MCR-1* resistance gene out of 100 *K. pneumoniae* isolates

MCR-1			
		Frequency	Percent
Colistin	Positive	4	4.%
	Negative	96	96%
	Total	100	100%

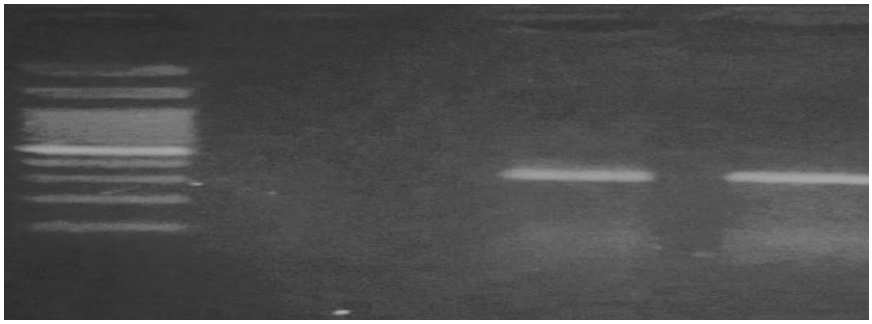

Figure 3.4: Detection of MCR-1 gene for Gel Electrophoresis, typical band size 309

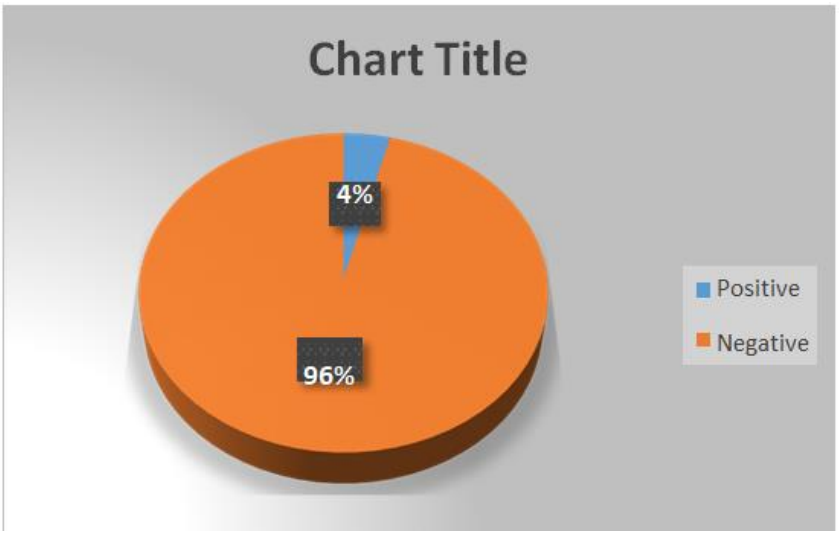

Figure 4.5: Percent of *MCR-1* gene positive in *K. pneumoniae* isolates

Table 3: Association between the presence of *MCR-1* gene and antimicrobial susceptibility test (AST) to Colistin

		MCR-1		Total	p-value=
		Negative	Positive		
Colistin	Resistance	8	3	11	0.000
		8%	3%	11%	
	Sensitive	88	1	89	
		88%	1%	89%	
Total		96	4	100	
		96%	4%	100%	

Thee isolates out of 4 (75%) were resistance to colistin and detected *MCR-1* gene by PCR and only one isolate (25%) of 89 colistin sensitive was detected *MCR-1* gene so that in statistically there was significant association between *MCR-1* gene and the resistance of Colistin the (p-value <0.05).

Table 4: Association between the MCR-1 gene and gender

		MCR-1		Total
		Negative	Positive	
Gender	Male	30	1	31
	Female	66	3	69
Total		96	4	100

The total of 100 isolated samples predominantly were female only 31% were male while 69% were female and the *MCR-1* were detected 3 out of 69 while only 1 out of 31 males were detected *MCR-1*, but there were no significant association between the MCR-1 gene and Gender the (p-value > 0.05).

Table 5: Association between the Colistin Resistance and gender

		Colistin Resistance		Total
		Sensitive	Resistance	
Gender	Male	28	3	31
	Female	61	8	69
Total		89	11	100

5. DISCUSSION

A total of one hundred Sudanese subjects were enrolled in this study to isolates of *k. pneumonia* from different hospitals in Khartoum state and confirmed by fermented lactose and produced smooth mucoid pink colonies. The patients ages were ranged from (17-40) years (Mean ± 27.4 SD = 6.598) and (31%) were male and (69 %) were female. All samples detected for sensitivity by using Kirby Bauer disc diffusion technique, the result show (89%) was sensitive to Colistin and (11%) were resistance. All 100 *K. pneumoniae* isolates investigated for the presence of Colistin resistance gene *(MCR-1)* using multiplex PCR. There were (4%) positive for MCR-1 gene and (96%) were negative.

Multiplex PCR was used to detect to Colistin resistance gene (MCR-1). In this study 4% of *K. pneumoniae* isolates were positive for MCR-1 gene which is agreement with previous study from Sudan by Zakaria A, Rhmtallah M, Abdo ZM in MCR-1 gene were positive (5 out of 185 samples) were *K. pneumoniae* [1]. and another previous study in Egypt in Zagazig University Hospital by Rabie RA, Abdallah AL *MCR-1* gene was positive (4.2%) *K. pneumoniae* which is slightly like my result [15]. And the previous study done by Shabban M, Fahim NA, Montasser K *et al*., in Mansoura University Hospital in Egypt, 50 isolates were detected *MCR-1* gene using PCR and 2(4%) isolated detected positive *MCR-1*gene one was *E. coli* and other was *K. pneumonia* [16].

In addition, the previous study by Mai M. Shogar [1], Mawada A. Ali [1], Misca A. Mohamed [1] and Mahadi H. Abdallah [2] the Out of 100 *Klebsiella Pneumoniae* Isolates 8 (8%) were colistin resistance, while the mcr-1 gene was detected in one sample (1%), that is slightly difference from our result. On the other hand, the most studies carried out in Asian countries and in European countries are slightly higher prevalence rate than in African Continent, in the middle east these previous study results found in six countries including Turkey (438), Iran (86), Saudi Arabia (24), United Arab Emirates (31), Kuwait (5), Israel (3) [21] the multicenter longitudinal study was performed in 28 hospitals in chine for 2017 were detected only one (<1%) Isolates of *K. pneumoniae* out of 571 isolates [7].

CONCLUSION & RECOMMENDATIONS

This study is conducted to determine the presence of colistin resistance *MCR-1* gene in Clinical samples of *Klebsiella pneumonia* Isolated from Khartoum State Hospitals. Out of 100 non duplicated *K. pneumoniae* 11(11%) were resistance to Colistin while 3(3%) of the resistance show positive *MCR-1* gene and only 1(1%) out of colistin sensitive samples was detected positive *MCR-1* gene. The total of detection of *MCR-1* gene in this study is 4(4%). The government must take some steps to stop spread of resistance bacteria, especially which is positive to mcr-1 gene. Antibiotics should not be used without doctor transcription, and must be used the right dose in the right time. Narrow spectrum antibiotics should be used rather than broad spectrum. Culturing and sensitivity testing of pathogenic bacteria should be taken before treatment begins.

REFERENCE

1. Zakaria, A., Rhmtallah, M., Abdo, Z. M., Ismail, M. F., Ali, A. H., & Hamad, M. N. M. (2020). Molecular detection of colistin resistance gene mcr-1 in Gramnegative rods isolated from hospitalized patients in Khartoum State. *Saudi Journal of Pathology and Microbiology*, 5(8), 380-384.
2. Hamed, Z. O., & Awni, A. A. (2017). Molecular Detection of B-Lactamase Gene [SHV] in Klebsiella Pneumoniae. Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J., & Dittrich, S. Antimicrobial resistance in Africa: a systematic review. *BMC infectious diseases*, 17(1), 1-7.
3. Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J., & Dittrich, S. (2017). Antimicrobial resistance in Africa: a systematic review. *BMC infectious diseases*, *17*(1), 1-17.
4. Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., ... & Wang, Y. (2018). Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. *Emerging microbes & infections*, *7*(1), 1-9.
5. Wyres, K. L., & Holt, K. E. (2018). Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Current opinion in microbiology*, *45*, 131-139.
6. Kadri, S. S. (2020). Key takeaways from the US CDC's 2019 antibiotic resistance threats report for frontline providers. *Critical care medicine*.
7. Aris, P., Robatjazi, S., Nikkhahi, F., & Marashi, S. M. A. (2020). Molecular mechanisms and prevalence of colistin resistance of Klebsiella pneumoniae in the Middle East region: a review over the last 5 years. *Journal of Global Antimicrobial Resistance*, *22*, 625-630.
8. Falagas, M. E., Kasiakou, S. K., & Saravolatz, L. D. (2005). Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clinical infectious diseases*, *40*(9), 1333-1341.
9. Tan, T. Y., & Ng, S. Y. (2006). The in-vitro activity of colistin in gram-negative bacteria. *Singapore medical journal*, *47*(7), 621.
10. Sarı, A. N., Süzük, S., Karatuna, O., Öğünç, D., Karakoç, A. E., Cizmeci, Z., ... & Gülay, Z. (2017). Results of a multicenter study investigating plasmid mediated colistin resistance genes (mcr-1 and mcr-2) in clinical Enterobacteriaceae ısolates from Turkey.
11. Bialvaei, A. Z., & Samadi Kafil, H. (2015). Colistin, mechanisms and prevalence of resistance. *Current medical research and opinion*, *31*(4), 707-721.
12. https://www.cdc.gov/drugresistance/solutions-initiative/stories/gene-reported-mcr.html web at 21/6/2021.
13. Wang, R., van Dorp, L., Shaw, L. P., Bradley, P., Wang, Q., Wang, X., ... & Balloux, F. (2018). The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nature communications*, *9*(1), 1-9.
14. Babiker, M. H. *Molecular Detection of Plasmids Mediated Colistin Resistance (MCR-1) Gene of Already Identified Klebsiella pneumoniae Isolated from Clinical Specimens in Khartoum State, Sudan*

(Doctoral dissertation, Sudan University of Science & Technology).

15. Rabie, R. A., & Abdallah, A. L. (2019). Plasmid mediated colistin resistant genes mcr-1 and mcr-2 among Escherichia coli and Klebsiella pneumoniae isolates at Zagazig University hospitals, Egypt. *Internal medicine*, 14:0-05.
16. Shabban, M., Fahim, N. A. E., Montasser, K., & El Magd, N. M. A. (2020). Resistance to colistin mediated by mcr-1 among multidrug resistant Gram negative pathogens at a tertiary care hospital, Egypt. *Journal of Pure and Applied Microbiology*, *14*(2), 1125-1132.
17. Sultan, A. M., & Seliem, W. A. (2018). Identifying risk factors for healthcare-associated infections caused by carbapenem-resistant acinetobacter baumannii in a neonatal intensive care unit. *Sultan Qaboos University Medical Journal*, *18*(1), e75.
18. Quan, J., Li, X., Chen, Y., Jiang, Y., Zhou, Z., Zhang, H., ... & Yu, Y. (2017). Prevalence of mcr-1 in Escherichia coli and Klebsiella pneumoniae recovered from bloodstream infections in China: a multicentre longitudinal study. *The Lancet Infectious Diseases*, *17*(4), 400-410.
19. Mccariney, M. (2006) Practical Medical Microbiology. 14th ed. New Delhi (India): Elsevier.
20. Cheesbrough, M. (2005). Distric laboratory Practice in Tropical Countries. Second Edition, Part 2. New York, Unied States; Cambridge university press.