

Content available at: <https://www.ipinnovative.com/open-access-journals>

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: <https://www.ijmmt.org/>

Original Research Article

Prevalence and molecular detection of *Klebsiella pneumoniae* isolated from children with infectious diarrhoea in AL-Ramadi cityFatima Sabri Al-Omar^{1*}¹Dept. of Biology, College of Science, University of Al-Anbar, Iraq

ARTICLE INFO

Article history:

Received 25-03-2024

Accepted 10-04-2024

Available online 22-06-2024

Keywords:

K pneumoniae

Gastroenteritis

PCR and Stool samples

ABSTRACT

Objectives: Infectious diarrhoea result from a wide range of bacteria, viruses and parasites. This condition is also identified as gastroenteritis, *Klebsiella pneumoniae* is a well-known as one of most common bacterial pathogens causing gastroenteritis. This study aims to estimate the incidence and identifying both the phenotypic and genotypic characterization of *Klebsiella pneumoniae* causing gastroenteritis in children under the age of five in al-Ramadi Maternity and Children Teaching Hospital.

Materials and Methods: Stool samples were collected for 106 children suffering from gastroenteritis, Cultural and microscopical approaches were used for *Klebsiella pneumoniae* selection, its characteristic features were confirmed using the Vitek2 compact system, anti-microbial sensitivity test, and biofilm production test. furthermore, DNA extracted, purification and Polymerase chain reaction (PCR) were accomplished for genotypic confirmation.

Results: In the presented research, stool samples were collected for 106 children suffering from gastroenteritis, and 100 samples were identified as source for bacterial gastroenteritis. The bacterium under consideration (*Klebsiella pneumoniae*) has a percentage of 30%. This isolate revealed resistance to Ceftazidime (80%), nalidixic acid (33%), amikacin (36%), Azithromycin (20%), vancomycin (10%), and Imipenem (6%). Likewise, four genes in *Klebsiella pneumoniae* isolate were studied via PCR and the results indicates htrA, iss, Mrka and rmpA were 15 (50%), 9 (30%), 6 (20%), 0 (0%) respectively. The results of biofilm production for *Klebsiella pneumoniae* exposed that 3 (10%) were strong, 10 (33%) moderate, 7 (24%) weak, and 10 (33%) non-producers.

Conclusions: The presented research displayed the bacterium under consideration (*Klebsiella pneumoniae*) has a higher resistance rate to the commonly antibiotics used for bacterial gastroenteritis. In addition, *Klebsiella pneumoniae* (under consideration) with high resistance to antibiotics showed resistance genes in PCR, in addition to strong biofilm production.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprint@ipinnovative.com

1. Introduction

Diarrheal illnesses represent a significant cause of sickness and death among children under five years old, accounting for approximately 500,000 annual fatalities and ranking third in the global burden of disability-adjusted life-years.¹ Many bacteria, viruses, and parasitic species can produce

diarrhea, which is typically a sign of digestive tract illnesses. Worldwide, diarrhea has a wide range of characteristics and a seasonal distribution. Each year, about 1.7 billion individuals suffer from diarrhea illness worldwide. It is considered as a major source of morbidity and mortality among children and represents a worldwide health concern.¹ Regional, social, and economic development, as well as geographic living practices, are intimately linked to the distribution and incidence of diarrheal diseases.^{2,3} The

* Corresponding author.

E-mail address: fat21s1002@uoanbar.edu.iq (F. S. Al-Omar).

majority of diarrhea-causing pathogens primarily transmit through the fecal-oral route. However, each pathogen employs unique infection mechanisms and diverse strategies to elicit clinical symptoms of diarrhea.⁴⁻⁷

Nevertheless, other research indicated that the predominant pathogenic agents linked to this disease were gut bacterial flora like *Escherichia* spp., *Salmonella* spp., *Shigella* spp., and *Klebsiella pneumoniae*.^{8,9} Coastal zones were the sites of more frequent observations of *Vibrio parahaemolyticus*.^{10,11}

It is challenging to identify its origin based on clinical symptoms, so laboratory testing is frequently crucial. The "gold standard" for treating bacterial diarrhea is still depends on the standard criteria of microbiology lab techniques for isolating the causative bacteria. However, this is affected by a variety of factors, including bacterial count in the sample, proficiency of lab operators, types of culture media and methods. Furthermore, the isolation will be significantly impacted by antibiotic treatment, producing falsely negative results. The lower estimation of bacteria in comparison with virus among sample obtaining may be due to the variation in the revealing sensitivity related with phenotypic and genotypic based approaches. Reducing turn around times and increasing detection method success rates have been attempted in an attempt to address issues with the medical remedy and epidemiologic assessments connected to diarrhoea infections. Among these efforts has been the creation of suitable assays depends on PCR based finding.^{12,13}

In general, molecular finding procedures have a higher sensitivity than the cultural techniques. Prior to strain isolation, it is assumed that sample screening using molecular techniques were more efficient than direct selection of isolate in all samples. This is due to earlier approach which focus on samples that tested positive for isolates. Finding employment costs would be decreased and the rate of positive detection would be raised. When it comes to bacterial diseases that require enrichment prior to isolate, like *Vibrio* and *Salmonella* species. More causative agents among the same samples will discovered through identifying the nucleic acids of supplemented culture, while molecular revealing based on stool nucleic acids may neglect certain samples which have lower bacterial counts.

2. Materials and Methods

2.1. Collection of stool samples

All specimens were taken from September 2022 to December 2022. The approval of parental consent for pediatric patients was obtained according to the guidelines of the Research Ethics Committee at Anbar University. Patients' information and history were recorded using a formal sheet questionnaire. Samples were collected from patients who attended al-Ramadi Maternity and

Children Teaching Hospital. Samples were isolated from the stool of children under five years of age, and then cultured on media blood agar, MacConkey agar, and S. S agar. Criteria for inclusion and exclusion in the study include factors such as age range of the children, clinical presentation of infectious diarrhea, diagnostic methods used for *Klebsiella pneumoniae* detection, and any comorbid conditions that may affect the study outcomes. This research has been approved by the Scientific Research Ethics Committee at Anbar University on^{11,12} with serial number 964783720431.

2.2. Isolation and identification of bacterial isolates

106 samples were collected from the excreta of infected children, and 30 isolates were identified as *Klebsiella pneumoniae*. Its characteristic features were confirmed using the Vitek2 compact system (BioMerieux, France).

2.3. Antimicrobial susceptibility test (AST)

The Kirby-Bauer method is a standardized system for testing the efficacy of antimicrobials against microorganisms. Pure isolated colonies (2-4) were inoculated in brain-heart broth (2ml) to obtain a bacterial suspension, which was compared with a 0.5 McFarland turbidity tube, Muller Hinton agar plates were inoculated using cotton swabs from that prepared suspension and incubated under standard condition. Anti-microbial discs were positioned upon the medium's surface. Then incubated at 37°C for 24 hours. For interpreting the results, the Clinical and Laboratory Institute's standards were followed (Clinical and Laboratory Institute Standards 2022).

2.4. Molecular identification

The PCR approach was used for revealing four virulence genetic factor (*htrA*, *rmpA*, *mrkA*, and *iss gene*) in the *Klebsiella pneumonia* isolates.

2.5. Extraction of bacterial DNA

The bacterial gDNA was recovered from bacterial isolates by the use of gDNA mini bacteria kit according to protocol from Favorgen, Taiwan. The pure gDNA solution was kept in -20 °C until use in the PCR.

2.6. PCR amplifications

The PCR amplification was used to identify the virulence genes present in *Klebsiella pneumonia* isolates; the primers used in PCR were lasted as seen in Table 1.

2.7. Biofilm test

The micro-titter plate method was used to detect the capability of *Klebsiella pneumonia* isolates for biofilm

Table 1: The primers used in current study. The key word F: Forward sequences, R: Reverse sequences.

Gene name		Primer sequence	Product size		TM (°C)
rmpA	F	5-CGCAGAAGTATTCCGTTGGT-3	967 bp	AB289644.	58
	R	5-TTTGTTAGCCGTGGATAATGG-3			
mrkA	F	5-AGTGCTTTCACCCCTCCT-3	862 bp	M55912.1	59
	R	5-GAGTGACTGGGGTGAGCAA-3			
iss	F	5-TGTCACATAGGATTCTGCCGTT-3	450 bp	NZ_RZLR01001650.1	60
	R	5-TTCACCCTCAGAGAGAGGCT-3			
htrA	F	5-CGTTCTGCCAGGATGGTTCT-3	1071 bp	AJ430233.1	59
	R	5-CCCCAATGATGACATCGCCT-3			

formation according to as following: Firstly, inoculum preparation (Turbidity standard) To obtain 1.5×10^8 CFU for each ml, few drops of freshly overnight broth culture were transferred into 5 ml sterile brain heart infusion medium, 0.5 Mc-Farland standard was used to evaluate the tube turbidity. Secondly, preparation of the 96-well micro plate (ELISA): Biofilm formation assessed as mentioned by,¹⁴ in which 200 μ l of the diluted cultures was loaded into the micro wells using a micropipette (three duplicates of each isolate), then 200 μ l of sterile BHI broth was added to 6 wells as a negative control. The plate was then sealed with a lid and aerobic incubation for eighteen hours at 37 °C. Thirdly, bacterial cultures were removed from all wells and washed twice with a sterile phosphate buffer saline (PBS). The plate was dried on filter paper by inverting the plate upside down. Then, 200 μ l of methanol was added in each well for 10 min that helps fixing the adhered bacterial cells (forming biofilm cells). Fourthly, the washing process was repeated carefully at room temperature to ensure the removal any excess of methanol from the wells. Finally, 200 μ l of the crystal violet stain (0.1 % w/v) was added in each well, the plate was left for 15 min at room temperature, excess stained was removed by washing three times with PBS, late to dry completely.

3. Result and Discussion

3.1. Isolation and identification of bacteria

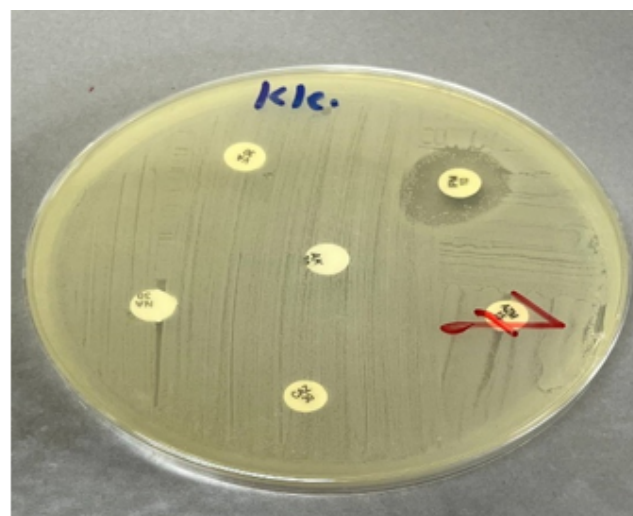
106 samples were collected from the discharge of children with gastroenteritis from al-Ramadi Maternity and Children Teaching Hospital. The samples infected with bacteria were identified and diagnosed using morphological and cultural criteria and the Vitek2 compact automated system, 30 isolates of the *Klebsiella pneumoniae* bacteria were obtained, with a percentage of (30%).

3.2. Antimicrobial susceptibility testing

The result showed a highest resistance of *Klebsiella pneumoniae* to Ceftazidime (80%), then amikacin (36%), nalidixic acid (33%), Azithromycin (20%), vancomycin (10%), Imipenem was found (6%) as shown in Figure 1 and Table 1.

Table 2: The antibiotic susceptibility of *Klebsiella pneumoniae*

Antibiotics	Resistant %	Intermediate %	Sensitive %
Ceftazidime	80	0	20
Amikacin	36	0	64
Nalidixic Acid	33	0	67
Azithromycin	20	0	80
Vancomycin	10	0	90
Imipenem	6	0	94

**Figure 1:** Antibiotic resistance of *Klebsiella pneumoniae* on Muller Hinton Agar.

3.3. Detecting of rmpA gene

In this study, none of the isolates showed a result for this gene (0%) as shown in Figure 2. The rmpA gene is a regulator of mucoid phenotype A (RmpA) gene found in *Klebsiella pneumoniae*, This gene is responsible for producing of the well-known capsular polysaccharide (CPS), as it is a sticky substance that helps these microbes to escape from the immunity defense procedure.¹⁵ The rmpA gene has been shown to be important for the virulence of *K. pneumoniae*. Studies have shown that mutants of *K. pneumoniae* that lack the rmpA gene are less virulent in animal models of infection. This is likely due to the fact

that the *rmpA* gene is required for the production of CPS.¹⁶



Figure 2: Gel electrophoresis for detection of *rmpA* gene (967 bp). 100-bp DNA ladder. PCR product were electrophoresis using agarose (1.5%) at 70V for 1.5 hr.

3.4. Detecting of *iss* gene

In this study, 9 isolates out of 30 showed results for this gene, at a rate of (30%) as shown in Figure 3. The *iss* gene is a member of the insertion sequence (*is*) family of mobile genetic elements, *iss* elements are little DNA sequences that have the ability to shift chromosomally. They are often found in association with antibiotic resistance genes. This gene is responsible for the insertion of other genetic elements, such as antibiotic resistance genes, into the *Klebsiella* chromosome. This can contribute to the development of multidrug-resistant (MDR) *Klebsiella* infections.¹⁷ It is also found in other bacteria for example *E. coli*, *S. enterica*. It is a common element in the genomes of these bacteria and have an important responsibility for bacterial development and alteration.



Figure 3: Gel electrophoresis for detection of *iss* gene (450 bp). 100-bp DNA ladder. PCR product were electrophoresis using agarose (1.5%) at 70V for 1.5 hr.

3.5. Detection of *mrkA* gene

In this study, 6 isolates out of 30 showed results for this gene, with a percentage of 20% as shown in Figure 4. The *mrkA* gene in *Klebsiella pneumoniae* is a one of MarR family members which are known as transcriptional regulators. MarR genes are responsible for regulating of genes collaborate in stress reply and antibiotic resistance. This gene has been shown to control the appearance of numerous genes that are responsible in antibiotic resistance, including the *acrAB* efflux pump and the *ompK36* porin.¹⁸ It has the capability of binding to the promoters' region in the *acrAB* efflux pump gene and repress it is expression. It is also binds to the promoters' region in the *ompK36* porin which activates its expression.¹⁹

3.6. Detecting of *htrA* gene

In this study, 15 isolates out of 30 showed results for this gene, with a percentage of 50%. as shown in Figure 5. The *htrA* gene is a protease which has an important function in the bacterium's virulence. It was firstly discovered in 1992 by researchers at the University of California, San Francisco (UCSF). It is a chromosomal gene of *K. pneumoniae*, and it encodes a protein that is involved in the degradation of proteins. It is also involved in the production of bacterial biofilm, which are populational of bacteria that are inserted

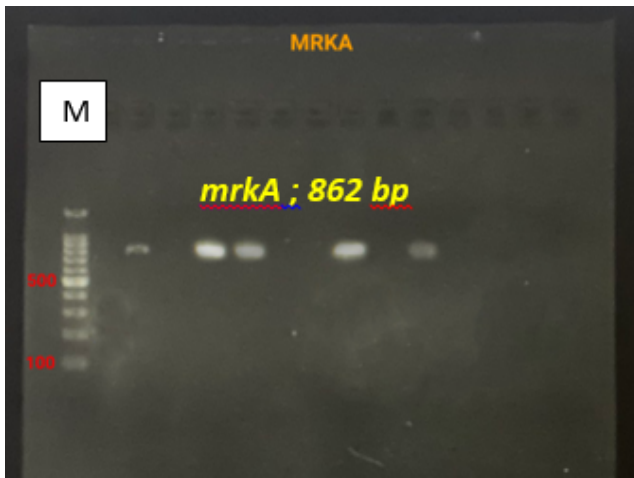


Figure 4: Gel electrophoresis for detection of *mrkA* gene (862 bp). M: 100-bp DNA Marker. PCR product were electrophoresis using agarose (1.5%) at 70V for 1.5 hr

in the matrix of extracellular polymeric substances (EPS). Bacterial biofilm is an important for the survival of *K. pneumoniae*, as they protect the bacteria from antimicrobial agents and other environmental stresses.²⁰ The *htrA* gene is a promising target for the development of new antimicrobial therapies for the treatment of *K. pneumoniae* infections. Inhibition of the *htrA* protein has been shown to reduce the virulence of *K. pneumoniae* in animal models, and it is likely that inhibitors of the *htrA* protein would also be effective in treating *K. pneumoniae* infections in humans.²⁰

3.7. Biofilm Formation ability by *Klebsiella pneumoniae*

This study examined *K. pneumoniae* ability to build biofilms. The findings revealed that 3 isolates (10%) had a great capacity for this creation, 10 isolates (33%) had a moderate capacity, 6 isolates (24%) were relatively weak producers of biofilm, and 10 isolates were non producers (33%) as shown in Figure 6. In the current work, *Klebsiella pneumoniae* ability to produce biofilms was assessed using an ELISA technique. Antibiotic resistance of this bacterium is partly due to its capability to construct biofilm on the biotics and the non-biotics surface, such as catheter and another medical means.²¹ It is found that the clinical isolates *K. pneumoniae* in the gastric system, isolates associated with pneumonia and urinary tract infections, a number of prerequisites for biofilm formation have been found.²² On other hand, a biofilm forms when planktonic bacteria adhere to a surface, initiating a series of events. The transition from planktonic to biofilm state is a multifaceted process influenced by genetic and environmental factors. Key genes implicated in *K. pneumoniae* biofilm formation encompass fimbriae, polysaccharides, quorum sensing (QS)

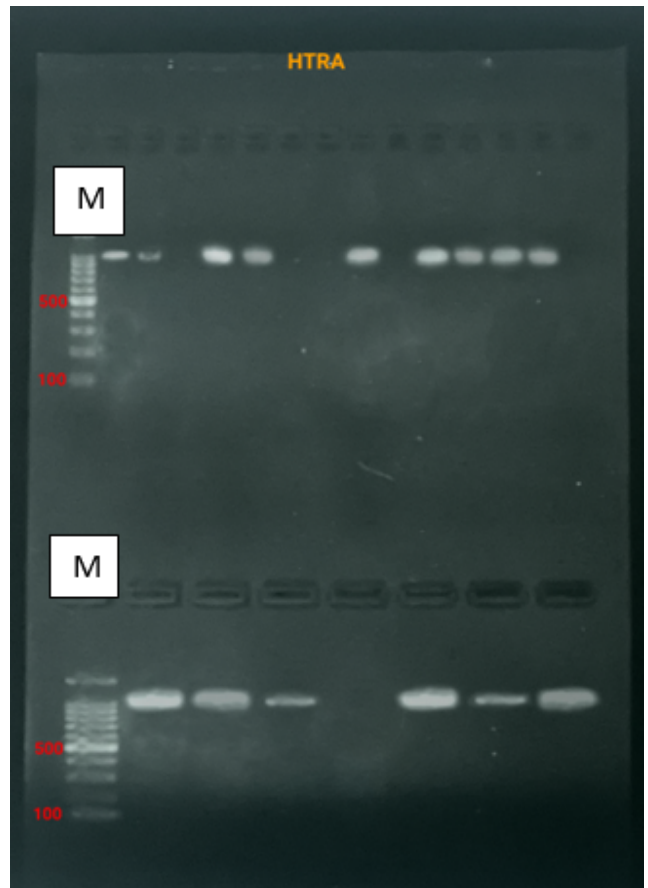


Figure 5: Gel electrophoresis for detection of *htrA* gene (1071 bp). M: 100-bp DNA Marker. PCR product were electrophoresis using agarose (1.5%) at 70V for 1.5 hr

systems, efflux pumps, among others.^{23,24}

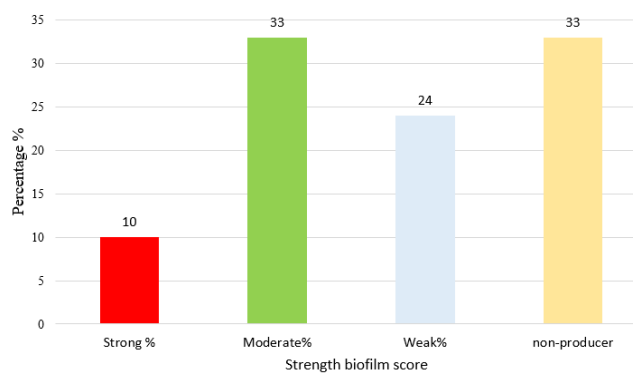


Figure 6: Strength of biofilm score

4. Conclusions

The research findings reveal that the bacterium under investigation, *Klebsiella pneumoniae*, exhibits elevated resistance levels to commonly prescribed antibiotics for

bacterial gastroenteritis. Moreover, the examined *Klebsiella pneumoniae* strains displaying heightened antibiotic resistance also demonstrated the presence of resistance genes in PCR analysis, along with robust biofilm formation.

5. Conflicts of Interest

The authors state that there is no conflict of interest.

6. Source of Funding

None.

7. Acknowledgments

The writers would like to thank University of Anbar, Iraq for its provision to the presented work.

References

- Atlas HE, Conteh B, Islam M, Jere KC, Omere R, Sanogo D, et al. Diarrhea case surveillance in the Enterics for Global Health Shigella surveillance study: epidemiologic methods. *Open Forum Infect Dis*. 2024;11(Suppl 1):6–16.
- Bier N, Schwartz K, Guerra B, Strauch E. Survey on antimicrobial resistance patterns in *Vibrio vulnificus* and *Vibrio cholerae* non-O1/non-O139 in Germany reveals carbapenemase-producing *Vibrio cholerae* in coastal waters. *Front Microbiol*. 2015;6:1179. doi:10.3389/fmicb.2015.01179.
- Letchumanan V, Yin WF, Lee LH, Chan KG. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. *Front Microbiol*. 2015;6:33. doi:10.3389/fmicb.2015.00033.
- Chavda VP, Vuppu S, Mishra T, Kamaraj S, Sharma N, Apostolopoulos V, et al. Combatting infectious diarrhea: innovations in treatment and vaccination strategies. *Expert Rev Vaccin*. 2024;23(1):246–65.
- Carroll SC, Castellanos ME, Stevenson RA, Henning L. Incidence and risk factors for travellers' diarrhoea among short-term international adult travellers from high-income countries: a systematic review with meta-analysis of cohort studies. *J Travel Med*. 2024;p. taae008. doi:10.1093/jtm/taae008.
- Zhu J, Wang Y, Li Y, Chen Y, Lu F. Risk factors of post-operative diarrhoea in patients with pancreatic cancer after neoadjuvant chemotherapy: A retrospective cohort study. *J Clin Nurs*. 2024;33(5):1777–85.
- Yu J, Jing H, Lai S, Xu W, Li M, Wu J, et al. Etiology of diarrhea among children under the age five in China: results from a five-year surveillance. *J Infect*. 2015;71(1):19–27.
- Qu M, Deng Y, Zhang X, Liu G, Huang Y, Lin C, et al. Etiology of acute diarrhea due to enteropathogenic bacteria in Beijing, China. *J Infect*. 2012;65(3):214–22.
- Zhu M, Cui S, Lin L, Xu B, Zhao J, Xia S, et al. Analysis of the aetiology of diarrhoea in outpatients in 2007, Henan province, China. *Epidemiol Infect*. 2007;141(3):540–8.
- Li Y, Xie X, Shi X, Lin Y, Mou J, Chen Q, et al. *Vibrio parahaemolyticus*, southern coastal region of China. *Emerg Infect Dis*. 2007;20(4):685–8.
- Zhang Y, Zhao Y, Ding K, Wang X, Chen X, Liu Y, et al. Analysis of bacterial pathogens causing acute diarrhea on the basis of sentinel surveillance in. *Jpn J Infect Dis*. 2006;67(4):264–8.
- Amar CFL, East CL, Gray J, Iturriza-Gomara M, Maclure EA, Mclauchlin J, et al. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993-1996). *Eur J Clin Microbiol Infect Dis*. 1993;26(5):311–23.
- Harrington SM, Buchan BW, Doern C, Fader R, Ferraro MJ, Pillai DR, et al. Multicenter evaluation of the BD max enteric bacterial panel PCR assay for rapid detection of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp.(*C. jejuni* and *C. coli*), and Shiga toxin 1 and 2 genes. *J Clin Microbiol*. 2015;53(5):1639–47.
- Abdullah EM, Mutlak ST. The incidence of Lower (UTI) according to the age and sex in Ramadi City. *J Fac Med Bagdad*. 2009;51(3):289–91.
- Hsu CR, Lin TL, Chen YC, Chou HC, Wang JT. The role of *Klebsiella pneumoniae* rmpA in capsular polysaccharide synthesis and virulence revisited. *Microbiology*. 2011;157(12):3446–7.
- Bakhtiari R, Javadi A, Aminzadeh M, Molaei-Aghae E, Shaffaghat Z. Association between presence of RmpA, MrkA and MrkD genes and antibiotic resistance in clinical *Klebsiella pneumoniae* isolates from hospitals in Tehran. *Iran J Public Health*. 2021;50(5):1009–16.
- Schwarz S, Cloeckert A, Roberts MC. Mechanisms of Bacterial Resistance to Antimicrobial Agents. *Microbiol Spectr*. 2005;6(1):73–98.
- Sundaramoorthy NS, Shankaran P, Gopalan V, Nagarajan S. New tools to mitigate drug resistance in Enterobacteriaceae - *Escherichia coli* and *Klebsiella pneumoniae*. *Crit Rev Microbiol*. 2023;49(4):435–54.
- Gerlach GF, Allen BL, Clegg S. Molecular characterization of the type 3 (MR/K) fimbriae of *Klebsiella pneumoniae*. *J Bacteriol*. 1988;170(8):3547–53.
- Cortés G, De Astorza B, Benedí VJ, Albertí S. Role of the htrA gene in *Klebsiella pneumoniae* virulence. *Infect Immun*. 2002;70(9):4772–6.
- Van Laar T, Chen T, Childers BM, Chen P, Abercrombie JJ, Leung KP, et al. Genome sequence of a multidrug-resistant strain of *Klebsiella pneumoniae*, BAMC 07-18, isolated from a combat injury wound. *Genome Announc*. 2014;2(6):e01230–14. doi:10.1128/genomeA.01230-14.
- Wu MC, Lin TL, Hsieh PF, Yang HC, Wang JT. Isolation of genes involved in biofilm formation of a *Klebsiella pneumoniae* strain causing pyogenic liver abscess. *PLoS One*. 2011;6(8):23500. doi:10.1371/journal.pone.0023500.
- Datta S, Nag S, Roy DN. Biofilm-producing antibiotic-resistant bacteria in Indian patients: a comprehensive review. *Curr Med Res Opin*. 2024;40(3):403–22.
- Li L, Gao X, Li M, Liu Y, Ma J, Wang X, et al. Relationship between biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* and updates on antibiofilm therapeutic strategies. *Front Cell Infect Microbiol*. 2024;14:1324895. doi:10.3389/fcimb.2024.1324895.

Author biography

Fatima Sabri Al-Omar, -

Cite this article: Al-Omar FS. Prevalence and molecular detection of *Klebsiella pneumoniae* isolated from children with infectious diarrhoea in AL-Ramadi city. *IP Int J Med Microbiol Trop Dis* 2024;10(2):108-113.