Detection of Several Carbapenems Resistant and Virulence Genes in Classical and Hyper-virulent Strains of *Klebsiella pneumoniae* Isolated from Hospitalized Neonates and Adults in Khartoum

Aalaa Mahgoub Albasha (loleta95227@outlook.com)
Sudan University of Science and Technology https://orcid.org/0000-0002-9501-7264
Esraa Hassan Osman
Sudan University of Science and Technology
Saga Abd Alhalim
Sudan university of science and technology
Elianz Alshaib
Sudan University of Science and Technology
Leena Al-Hassan
Brighton and Sussex Medical School
Hisham N Altayb
King Abdulaziz University

Research article

Keywords: K. pneumoniae, MDR, XDR, hvKp, nosocomial infection and, Sudan

Posted Date: May 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-26141/v1

Version of Record: A version of this preprint was published at BMC Research Notes on July 1st, 2020. See the published version at https://doi.org/10.1186/s13104-020-05157-4.

Abstract

Background

Carbapenems resistant hypervirulent strains of *Klebsiella pneumoniae* are one of the most critical organisms that cause fatal nosocomial infections. This study aimed to detect and characterize *K. pneumoniae* virulence genes (*mrk*D, *ent*B, *rmp*A, *K*2, *kfu*, and *mag*A) and carbapenem resistant (bla_{NDM}, bla_{IMP}, bla_{OXA-48}, and bla_{KPC}) genes

Methods

Sixty *K. pneumoniae* strains were isolated from urine, blood, wound swab, and sputum samples, in two age groups: neonates and adults. String test was used to detect hypervirulent strains. Susceptibility testing for a wide range of antibiotics was performed on all isolates. DNA was extracted by the guanidine chloride method, then multiplex PCR was used for the detection of carbapenem-resistance and virulence genes.

Results

Seventy percent of the isolates were resistant to ceftazidime and 8% to imipenem, 35% were multi-drug resistant, and 7% extensively drug-resistant, all neonatal blood isolates (n = 15) were resistant to ceftazidime. *entB* was the most predominant virulence gene (93.3%), followed by *mrkD* (78.3%), *kfu* (60%), *K2* (51.7%), *magA* (18.3%) and *rmpA* (5%). bla_{OXA-48} was the most predominant carbapenem-resistant gene (68.3%), followed by bla_{NDM} (10%), bla_{KPC} (8.3%), and bla_{IMP} (3.3%). Eight hyper-virulent strains were positive for bla_{OXA-48} and two for bla_{NDM} genes. The study concluded that there is a high rate of carbapenems resistant genes in hyper-virulent strains of *K. pneumoniae* isolated from hospitalized patients.

Conclusion

The study reported for the first time in Sudan presence of carbapenems resistant genes in hyper-virulent strains of *K. pneumoniae* isolated from hospitalized patients. Presence of MDR and XDR strains of *K. pneumoniae* in neonatal ward in some Sudanese hospitals.

Background

Klebsiella pneumoniae (K. pneumoniae) is a non-motile, capsulated gram-negative rod about 1–2 μm long, and is a facultative anaerobe [1]. It is a common cause of urinary tract, soft-tissue, and central nervous system infections, in addition to endocarditis, and cases of severe bronchopneumonia, sometimes with chronic destructive lesions and multiple abscess formation in the lungs. In many cases, localized infections lead to bacteremia [1].

There are two types of *K. pneumoniae* strains “classic” (cKp), usually non-virulent and drug-resistant gene producer and usually associated with hospital infections, while the other type is a hypervirulent (hvKp) drug-sensitive strain [2]. *K. pneumoniae* possesses different virulence and antimicrobial resistance genes associated with various clinical conditions. The vast majority of *K. pneumoniae* strains produce a capsular polysaccharide (CPS), which is considered the most crucial virulence factor [3], and its expression is enhanced by the regulator of mucoid phenotype A (*rmp*A) gene, which has an association with community-acquired pyogenic liver abscess (CA-PLA) [4]. Klebsiella ferric uptake (*kfu*) gene is a putative pathogenic gene associated with purulent tissue infections, capsule formation, and virulent hypermucoviscosity phenotype [5], mucoviscosity-associated gene-A (*mag*A) in *K. pneumoniae* is associated with pyogenic liver abscess [6]. Most strains of *K. pneumoniae* have K markers; K1 and K2 were found to be significantly more resistant to phagocytosis and associated with nosocomial infection than non-K1/K2 isolates [7]. *mrk*D (mannose resistant Klebsiella like hemagglutinin-D) gene mediates binding to extracellular matrices and likely to have a role in the colonization of damaged epithelial surfaces [8]. Enterobactin-B (*ent*B) is considered as one of the siderophore systems, which has a high affinity to bind to Fe [9].

Carbapenemase enzymes of *K. pneumoniae* can resist most β-lactam-ring-containing antibiotics, including carbapenems, and thus conferring resistance to these drugs [10]. Ambler molecular class A *K. pneumoniae* carbapenemase (KPC), class B; Verona integron metallo-betalactamases types (VIM), Imipenemase (IMP) and New Delhi metallo-betalactamase (NDM) and class D oxacillinase-48 (OXA-48) are frequently isolated from severe hospital infections [11]

Carbapenem resistant hypervirulent strains of *K. pneumonia* are one of the most important organisms that cause fatal nosocomial infections [12]. Recently, increasing reports of resistance to carbapenem in healthcare-associated with *K. pneumonia* infections have been documented in Sudan [13] [14] [15]. The mortality rate of carbapenem-resistant *K. pneumoniae* bacteremia could reach 50% of cases [16].

However, up to date, there is no published data in Sudan about the distribution and epidemiology of various types of Carbapenemases and virulence genes on hvKp and cKp strains circulating in Khartoum hospitals. This information is of great importance to understand their local epidemiology and to establish eradication and prevention procedures. Thus this study was conducted to detect and to characterize the common virulence and carbapenem-resistant genes of hvKp and cKp strains isolated from hospitalized patients in different hospitals in Khartoum state.

Results

Demographic data

Sixty *K. pneumoniae* isolates were obtained from different hospitals in Khartoum State, 27 (45%) were from females, and 33 (55%) from males, 37 (61.7%) were from urine, 15 (25%) were from neonatal sepsis, and 11 (18%) were from elderly patients (Table 2).

String Test

Out of sixty *K. pneumoniae* isolates, 9 (16%) were hypermucoviscous, and 47 (84%) isolates were classic.

Susceptibility Test Results

Most strains, 42 (70%), were resistant to ceftazidime and only 5(8%) resistant to imipenem (Table 2). Multi-drug resistant isolates were detected in 12 of urine isolates, 7 of blood, and 2 of wound swab isolates. Three neonatal blood isolates and one adult wound swab were showed extensively drug-resistant.

PCR Results

Detection of K. pneumoniae **carbapenem-resistant and virulence genes**

Eighty percent (48/60) of isolates were positive for carbapenem-resistant genes: 68.3% (41/60) were positive for $bla_{\text{OXA-48}}$ gene, 10% (6/60) were positive for bla_{NDM} gene, 8.4% (5/60) were positive for bla_{KPC} gene, and 3.3% (2/60) were positive for bla_{IMP} gene. One neonatal blood isolate possesses three carbapenem-resistant genes (bla_{KPC}, $bla_{\text{OXA-48}}$, and bla_{IMP}), six isolates possess two genes (four possess $bla_{\text{OXA-48}}$ and bla_{NDM}, two possess $bla_{\text{OXA-48}}$ and bla_{KPC}), and thirty-nine isolates possess one gene (34 $bla_{\text{OXA-48}}$, two bla_{NDM}, *two* bla_{KPC}, and *one* bla_{IMP}) and the remaining (12) were negative for all carbapenem-resistant genes. Eight hyper-virulent strains were harboring $bla_{\text{OXA-48}}$ and two harboring bla_{NDM} genes (Table 4) (Figs. 1, 2, and 3).

For virulence genes *mrk*D detected in 47 (78.3%) isolates, *ent*B in 56(93.3%), *rmp*A in 3(5%), *K2* in 31(51.7%), *kfu* in 36 (60%) and *mag*A in 8(13.3%) isolates. (Table 3) (Fig. 4).

There was no significant statistical association between the presence of virulence genes and carbapenems resistant genes (p-value was < 0.05). A total of 92% (43/47) of *mrk*D gene positive isolates were positive for one or more carbapenem resistant genes. There was strongly significant association between the presence of *mrkD* and *entb* genes (p-value = 0.0005), they were co-existed in 46 isolates.

Discussion

In this study, eight hyper-virulent strains of *K. pneumoniae* were reported positive for carbapenems resistant genes (*OXA-48* and *NDM)*. The presence of these strains in the clinical setting will complicate clinical practice and will cause fatal nosocomial infections [12]. Although antimicrobial-resistant hvKP strains are rarely reported worldwide [17–19], but here in Sudan they appear to be more prevalent.

Eight neonatal blood isolates were multidrug-resistant, and three of them were extensively resistant to all antibiotics that were used. Consequently the emergence of MDR pathogens would increase the mortality and morbidity and prolong hospitalization and cost of treatment [20].

All neonatal blood isolates (15) were resistant to ceftazidime. Ceftazidime-resistant *Klebsiella pneumoniae* (CRKP) in the pediatric oncology units of some Sudanese hospitals may be the cause of recent reports of high mortality rate associated with *K. pneumoniae* infections among this group in different Sudanese hospitals [21]. According to Schiappa [22], high resistance rates to ceftazidime could be due to the presence of a predominant enzyme (TEM-10) responsible for ceftazidime resistance in bloodstream isolates.

The isolates showed varying degrees of resistance to the other antibiotics; ciprofloxacin 30%, gentamicin 40%, and ceftazidime (70%). Resistance to these antibiotics may also be due to the presence of Extended-Spectrum Beta-lactamases (ESBLs) and other mechanisms like efflux pumps and porin mutations [23], which were not covered in this study.

Although chloramphenicol is used as a treatment of choice for MDR gram-negative bacilli bacteria [24], 38% of our isolates were resistant to it, which may be caused by transferable enzymatic resistance to aminoglycosides, thatis common in some hospitals [25].

In the current study, 94% (51/54) of the isolates harboring carbapenem-resistant genes were phenotypically susceptible to imipenem. This confirms what Walsh [26] said that this gene is not stable and relies upon other synergistic mechanisms to mediate resistance against carbapenems. In addition to imipenem, other antibiotics were analyzed in this study.

Of 48 *K. pneumoniae* isolates detected of having carbapenem-resistant genes, 10 had multiple genes co-occurring. This finding agrees with Ali & Omer [27] and Satir [28], which showed a multiplicity of genes in their isolates.

A total of 80% (4/5) of *KPC* and 100% (2/2) of *IMP* genes were positive among infant blood samples, and this may be due to organisms harboring these genes have a high ability to cause systemic infections, particularly in immunocompromised patients [29].

In this study, we found the essential gene for *K. pneumoniae* siderophores system *entB* gene is positive in 93.3% of all *K. pneumoniae* isolates, the rest (6.7%) of isolates that do not possess *entB* may contain other enterobactin (*ent*A, C, D, E or F), or other siderophores systems like yersiniabactin or aerobactin as reported by Lawlor [20]. Furthermore, *mrk*D gene is presented in 78.3% of the isolate. This gene has been found to be important in adhesion as reported by

Chen et al. (2012)[29]. The *rmp*A gene was detected in 5% of isolates, in contrast with Aljanaby and Alhasani [20] who found the *rmp*A gene present in 62.5% of *K. pneumoniae* isolates. This difference may be attributed to its mode of inheritance as plasmid-mediated as mentioned by [20] indicating the limited spread of this gene in our local strains in Sudan.

The capsular serotype gene *K2* was present in 51.7% of isolates; the rest of isolates may contain other capsular serotypes, as mentioned by Ho [30]. This study showed that *K*2 is present in 80% of hypermucoviscous strains, indicating that there is a relationship between the presence of *K*2 gene and hypermucoviscous strains of *K. pneumoniae*, which is in agreement with the study by Guo [31] which found that *K*2 is the most common capsular serotype in hypermucoviscous strain. In contrast to other studies [20, 32] [33, 34], which found *K1* was the most prevalent capsular serotype among hypermucoviscous *K. pneumoniae*.

The *kfu* gene (which codes for an iron uptake system) was present in 60% of isolates. The study showed no association between the presence of *kfu* gene and hypermucoviscosity. This finding disagrees with previous studies [20, 35, 36], which showed that *kfu* gene is associated with hypermucoviscosity phenotype; which may be attributed to diversity in geographical locations of studies.

The *mag*A gene was found in 13.3% of isolates. The study showed no association between the presence of *magA* gene and hypermucoviscous strains. Although this gene is highly essential for *K. pneumoniae*, which confirms bacterial mucoviscosity, its prevalence among local isolates is not high, suggesting that other genes play a role in the formation of mucoviscosity [20].

Conclusion

The study reported for the first time in Sudan presence of carbapenems resistant genes in hyper-virulent strains of *K. pneumoniae* isolated from hospitalized patients. Presence of MDR and XDR strains of *K. pneumoniae* in neonatal ward in some Sudanese hospitals.

Methods

A total of 60 isolates of *Klebsiella pneumoniae* were obtained from hospitalized patients in different hospitals in Khartoum state (Royal Care International Hospital, Al-Baraha Hospital, Soba University Hospital, Al-Amal National Hospital, East Nile Model Hospital, Omdurman Medical Military Hospital, and Police Hospital) during the period from January 2017 to March 2017 They were isolated from the urine (37), blood (14), respiratory tract (4) and wound infection (5).

Bacterial Identification

The isolates were identified by gram stain, standard biochemical methods (urease test, indole test, and carbohydrates fermentation test, motility test, and citrate utilization test) [37] [38], and by *K. pneumoniae* species-specific primers (Table 1) targeting the 16S rRNA gene. Antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method on Mueller Hinton agar using the following antibiotics; ciprofloxacin (5mcg), gentamicin (10mcg), ceftazidime (30mcg), imipenem (10mcg), and chloramphenicol (30) (HiMedia Laboratories Pvt. Ltd. Mumbai, India) [39]. *E. coli* ATCC 25922 and *K. pneumonia* (ATCC 700603) were used as quality control strains.

Capsule stain was used to detect capsule [40]. String test was used to differentiate between hvKp and cKp strains: if the grown colonies of *K. pneumoniae* form a string > 5 mm in length using a sterile loop, this demonstrates the hypermucoviscosity phenotype [41].

DNA Extraction

DNA was extracted using the guanidine chloride method [42]. Several colonies from overnight nutrient agar growth were washed with normal saline (NS), followed by the addition of 2 mL lysis buffer, 5 µl proteinase K, 1 ml guanidine chloride, and 300 µl ammonium acetate. The suspension was incubated overnight at 37 °C, on the next day, 2 ml of chilled chloroform was added. After centrifugation, the transparent upper layer was collected in a new 15 ml tube, then 10 ml of absolute cold ethanol was added to enhance precipitation of DNA. The pellet was washed with 70% ethanol and allowed to dry. The pellet was suspended with nuclease-free water and quantified using gel electrophoresis. The DNA samples were stored at -80 °C until used for PCR.

Detection Of Virulent And Resistant Genes

A primer sets targeting virulence and carbapenem-resistant genes of *K. pneumoniae* are shown in Table 1. The primers were dissolved according to manufacturer guidelines to prepare 10 pmol/µl in all PCR reactions.

PCR Conditions

PCR was carried out in a 20 µl volume using the Maxime PCR PreMix kit (iNtRON Biotechnology, Seongnam, Korea), 1 µl of each forward and reverse primer (10 pmol/µL), 2 µl of DNA, and then the volume was completed to 20 µl by distilled water. Four multiplex and single reaction PCR protocols were used for amplification of 16S rRNA, resistant and virulence genes, the initial melting temperature for all was 95 °C for 5 minutes and a final extension was at 72 °C for 10 minutes. Details of annealing temperatures are listed in Table 1.

Data analysis:

Data were analyzed by Statistical package for the social sciences (SPSS) version 16. Chi-square test was used for the analysis of the relationship between variables. A *p*-value of < 0.05 was considered statistically significant

Abbreviations

Abbreviation

bla

β-lactamase

CA-PLA

community-acquired pyogenic liver abscess

cKp

classic *K. pneumoniae*

CLSI

Clinical and Laboratory Standards Institute

CPS

capsular polysaccharide

***ent*B**

Enterobactin B

ESBL

extended- spectrum β- lactamase

hvKp

Hyper-virulent *Klebsiella pneumoniae*

IPM

Imipenem

kfu

Klebsiella Ferric Uptake

KPC

Klebsiella pneumoniae carbapenemase

OXA-48

Oxacillinase 48

***mag*A**

Mucoviscosity-Associated Gene A

MDR

Multi Drug Resistant

***mrk*D**

Mannose Resistant Klebsiella like hemoagglutinin D

NDM

New Delhi metallo

PCR

polymerase chain reaction

***rmp*A**

Regulatory of Mucoid Phenotype A

SPSS

Statistical Package for the Social Sciences

XDR

Extensively drug-resistant

Declarations

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request**.**

Ethics declarations:

Ethics approval and consent to participate

The research was approved by institutional ethics committee of deanship of scientific research, Sudan University of Science and Technology No: DSR-IEC3-01-07.

Verbal consent was obtained from participants (in case of neonates parental consent was obtained).
Written consent was waived by the ethical committee Of Sudan University of Science and Technology, meeting No (SUST/DSR/1EC/EA2/2017) Date (07/01/2017). Because we are using a previously collected human bio-specimens with limited data.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

None

Authors' contributions

AMA, HNA, SAA, EFA and EHO designed the study, AMA, SAA, EFA and EHO performed the experiments, HNA, AMA, and SAA analyzed the data, HNA, AMA and LAH wrote the manuscript, all the authors approved the final version of the manuscript.

Acknowledgements

Not applicable

References

1. Greenwood D, Slack RC, Barer MR, Irving WL: **Medical Microbiology E-Book: A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. With STUDENT CONSULT Online Access**: Elsevier Health Sciences; 2012.
2. Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, Skvortsova NN. **Virulence factors and antibiotic resistance of Klebsiella pneumoniae strains isolated from neonates with sepsis**. *Frontiers in medicine* 2018, 5.
3. Huynh DTN, Kim A-Y, Kim Y-R. Identification of Pathogenic Factors in Klebsiella pneumoniae Using Impedimetric Sensor Equipped with Biomimetic Surfaces. Sensors. 2017;17(6):1406.
4. Hsu C-R, Lin T-L, Chen Y-C, Chou H-C, Wang J-T. The role of Klebsiella pneumoniae rmpA in capsular polysaccharide synthesis and virulence revisited. Microbiology. 2011;157(12):3446–57.
5. Aljanaby A. Role of rmpA, wabG, uge, Ycfm, fimh1, EntB, Ybt-irp2 and kfu genes in pathogenicity of Klebsiella pneumoniae: An overview. International Journal of ChemTech Research International. 2017;10(1):391–8.

6. Yeh K-M, Kurup A, Siu L, Koh Y, Fung C-P, Lin J-C, Chen T-L, Chang F-Y, Koh T-H. Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for Klebsiella pneumoniae liver abscess in Singapore and Taiwan. J Clin Microbiol. 2007;45(2):466–71.
7. Abdul-Razzaq MS, Al-Khafaji JKT, Al-Maamory E. Molecular characterization of capsular polysaccharide genes of Klebsiella pneumoniae in Iraq. Int J Curr Microbiol App Sci. 2014;3(7):224–34.
8. Jagnow J, Clegg S. Klebsiella pneumoniae MrkD-mediated biofilm formation on extracellular matrix-and collagen-coated surfaces. Microbiology. 2003;149(9):2397–405.
9. Lawlor MS, O'Connor C, Miller VL. Yersiniabactin is a virulence factor for Klebsiella pneumoniae during pulmonary infection. Infect Immun. 2007;75(3):1463–72.
10. Lee C-R, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Frontiers in microbiology. 2016;7:895.
11. Al-Zahrani IA, Alasiri BA. The emergence of carbapenem-resistant Klebsiella pneumoniae isolates producing OXA-48 and NDM in the Southern (Asir) province, Saudi Arabia. Saudi Med J. 2018;39(1):23.
12. Zhang Y, Zhao C, Wang Q, Wang X, Chen H, Li H, Zhang F, Li S, Wang R, Wang H. High prevalence of hypervirulent Klebsiella pneumoniae infection in China: geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob Agents Chemother. 2016;60(10):6115–20.
13. Adam MA, Elhag WI. Prevalence of metallo-β-lactamase acquired genes among carbapenems susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. BMC Infect Dis. 2018;18(1):668.
14. Dahab R, Ibrahim AM, Altayb HN: **Phenotypic and genotypic detection of carbapenemase enzymes producing gram-negative bacilli isolated from patients in Khartoum State**. *F1000Research* 2017, **6**.
15. Copur Cicek A, Saral A, Ozad Duzgun A, Yasar E, Cizmeci Z, Ozlem Balci P, Sari F, Firat M, Altintop YA, Ak S, et al. Nationwide study of Escherichia coli producing extended-spectrum beta-lactamases TEM, SHV and CTX-M in Turkey. J Antibiot. 2013;66(11):647–50.
16. Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R, Schlaeffer F, Sherf M. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. Infection Control Hospital Epidemiology. 2009;30(10):972–6.
17. Su S-C, Siu L, Ma L, Yeh K-M, Fung C-P, Lin J-C, Chang F-Y: **Community-acquired liver abscess caused by serotype K1 Klebsiella pneumoniae with CTX-M-15-type extended-spectrum β-lactamase**. *Antimicrobial agents and chemotherapy* 2008, **52**(2):804–805.
18. Cheng N-C, Yu Y-C, Tai H-C, Hsueh P-R, Chang S-C, Lai S-Y, Yi W-C, Fang C-T. Recent trend of necrotizing fasciitis in Taiwan: focus on monomicrobial Klebsiella pneumoniae necrotizing fasciitis. Clinical infectious diseases. 2012;55(7):930–9.
19. Li W, Sun G, Yu Y, Li N, Chen M, Jin R, Jiao Y, Wu H. Increasing occurrence of antimicrobial-resistant hypervirulent (hypermucoviscous) Klebsiella pneumoniae isolates in China. Clinical infectious diseases. 2013;58(2):225–32.
20. Behera B, Das A, Mathur P, Kapil A, Gadepalli R, Dhawan B. Tigecycline susceptibility report from an Indian tertiary care hospital. Indian Journal of Medical research. 2009;129(4):446.
21. Abdelaziz M, Hamadalnil Y, Hashim O, Bashir T, Mahjoub E. **Microbiological Profile of Neonatal Sepsis at a Maternity Hospital in Omdurman, Sudan**. *Sudan Journal of Medical Sciences (SJMS)* 2019:45–51.
22. Schiappa DA, Hayden MK, Matushek MG, Hashemi FN, Sullivan J, Smith KY, Miyashiro D, Quinn JP, Weinstein RA, Trenholme GM. Ceftazidime-resistant Klebsiella pneumoniae and Escherichia coli bloodstream infection: a case-control and molecular epidemiologic investigation. J Infect Dis. 1996;174(3):529–36.
23. Singh-Moodley A, Perovic O. Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in Enterobacteriaceae in South Africa. BMC Infect Dis. 2016;16(1):536.
24. Yu W-L, Ko W-C, Cheng K-C, Lee H-C, Ke D-S, Lee C-C, Fung C-P, Chuang Y-C. Association between rmpA and magA genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. Clinical infectious diseases. 2006;42(10):1351–8.
25. Mekki AH, Hassan AN, Elsayed DEM. Extended spectrum beta lactamases among multi drug resistant Escherichia coli and Klebsiella species causing urinary tract infections in Khartoum. African Journal of Bacteriology Research. 2010;2(3):18–21.
26. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents. 2010;36:8–14.
27. Ali AHI, Al Fadhil AO. **Journal of Clinical Review & Case Reports**.
28. Satir S, Elkhalifa A, Ali M, El Hussein A, Elkhidir I: **Detection of Carbepenem resistance genes among selected Gram Negative bacteria isolated from patients in-Khartoum State, Sudan. Clin Microbiol 5: 266. doi: 10.4172/2327-5073.1000266 Page 2 of 4 Clin Microbiol, an open access journal ISSN: 2327–5073 Volume 5• Issue 6• 1000266**. Figure 2016, **2**:3.
29. Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of Klebsiella pneumoniae carbapenemases (KPC) resistance. Infection drug resistance. 2012;5:133.
30. Ho J-Y, Lin T-L, Li C-Y, Lee A, Cheng A-N, Chen M-C, Wu S-H, Wang J-T, Li T-L, Tsai M-D. Functions of some capsular polysaccharide biosynthetic genes in Klebsiella pneumoniae NTUH K-2044. PLoS One. 2011;6(7):e21664.
31. Guo Y, Wang S, Zhan L, Jin Y, Duan J, Hao Z, Lv J, Qi X, Chen L, Kreiswirth BN. Microbiological and clinical characteristics of hypermucoviscous Klebsiella pneumoniae isolates associated with invasive infections in China. Frontiers in Cellular Infection Microbiology. 2017;7:24.
32. Liu YM, Li BB, Zhang YY, Zhang W, Shen H, Li H, Cao B. Clinical and molecular characteristics of emerging hypervirulent Klebsiella pneumoniae bloodstream infections in mainland China. Antimicrob Agents Chemother. 2014;58(9):5379–85.
33. Qu T-t, Zhou J-c, Jiang Y, Shi K-r, Li B, Shen P. Wei Z-q, Yu Y-s: **Clinical and microbiological characteristics of Klebsiella pneumoniae liver abscess in East China**. BMC Infect Dis. 2015;15(1):161.

34. Yan Q, Zhou M, Zou M, Liu W-e. Hypervirulent Klebsiella pneumoniae induced ventilator-associated pneumonia in mechanically ventilated patients in China. European Journal of Clinical Microbiology Infectious Diseases. 2016;35(3):387–96.
35. Hsieh P-F, Lin T-L, Lee C-Z, Tsai S-F, Wang J-T. Serum-induced iron-acquisition systems and TonB contribute to virulence in Klebsiella pneumoniae causing primary pyogenic liver abscess. J Infect Dis. 2008;197(12):1717–27.
36. Ma L-C, Fang C-T, Lee C-Z, Shun C-T, Wang J-T. Genomic heterogeneity in Klebsiella pneumoniae strains is associated with primary pyogenic liver abscess and metastatic infection. The Journal of infectious diseases. 2005;192(1):117–28.
37. Cheesbrough M: **District laboratory practice in tropical countries**: Cambridge university press; 2006.
38. Leboffe MJ, Pierce BE: **A photographic atlas for the microbiology laboratory**: Morton Publishing Company; 2012.
39. CLSI C. **Performance standards for antimicrobial susceptibility testing**. *Clinical Lab Standards Institute* 2016.
40. McKinney RE. Staining bacterial polysaccharides. J Bacteriol. 1953;66(4):453.
41. Aljanaby AAJ, Alhasani AHA. Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. African Journal of Microbiology Research. 2016;10(22):829–43.
42. Sabeel S, Salih MA, Ali M, EL-Zaki S-E, Abuzeid N, Elgadi ZAM, Altayb HN, Elegail A, Ibrahim NY, Elamin BK. **Phenotypic and genotypic analysis of multidrug-resistant Mycobacterium tuberculosis isolates from Sudanese patients**. *Tuberculosis research and treatment* 2017, 2017.
43. Mahmudunnabi G, Momtaz F, Foysal MJ, Rahman MM, Islam K. Molecular detection and PCR-RFLP analysis using Pst1 and Alu1 of multidrug resistant Klebsiella pneumoniae causing urinary tract infection in women in the eastern part of Bangladesh. Journal of Genetic Engineering Biotechnology. 2018;16(1):77–82.
44. Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, Kassis-Chikhani N, Arlet G, Decré D. Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of Klebsiella pneumoniae. J Clin Microbiol. 2014;52(12):4377–80.
45. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F: **Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania**. *BioMed research international* 2014, **2014**.

Tables

Table 1
Primers sequences and PCR protocols used in this study

Protocols	Temperature cycling	Marker	Sequence (5 – 3')	Amplicons size (bp)	References
1st	35 cycles at 94 °C for 30 s, 58 °C for 90 s and 72 °C for 90 s	16 s rRNA	F. ATTTGAAGAGGTTGCAAACGAT	130	[43]
			R.TTCACTCTGAATTTTCTTGTGTTC		
2nd	30 cycles at 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 60 s	*mrkD*	F. AAGCTATCGCTGTACTTCCGGCA	340	* [44]
			R. GGCGTTGGCGCTCAGATAGG		
		entB	F. GTCAACTGGGCCTTTGAGCCGTC	400	
			R. TATGGGCGTAAACGCCGGTGAT		
		rmpA	F. CATAAGAGTATTGGTTGACAG	461	
			R. CTTGCATGAGCCATCTTTCA		
		K2	F. CAACCATGGTGGTCGATTAG	531	
			R. TGGTAGCCATATCCCTTTGG		
		kfu	F. GGCCTTTGTCCAGAGCTACG	638	
			R. GGGTCTGGCGCAGAGTATGC		
		magA	F. GGTGCTCTTTACATCATTGC	1283	
			R. GCAATGGCCATTTGCGTTAG		
3rd	35 cycles at 94 °C for 20 s, 56 °C for 10 s, 72 °C for 20 s	*NDM*	F. GGTTTGGCGATCTGGTTTTC	521	[45][45][45] [45][25][25]
			R. CGGAATGGCTCATCACGATC		
		IMP	F. TTGACACTCCATTTACAG	232	
			R. GATTGAGAATTAAGCCACTCT		
4th	35 cycles at 94 °C for 45 s, 52 °C for 1 minute, and 72 °C for 1 minute	*KPC*	F. CATTCAAGGGCTTTCTTGCTGC	498	
			R. ACGACGGCATAGTCATTTGC		
		OXA-48	F. GCTTGATCGCCCTCGATT	281	
			R. GATTTGCTCCGTGGCCGAAA		

Abbreviations: s = second, F = Forward, R = Reverse, bp = base pair

*Annealing time changed from 90 s to 45 s.

Table (2) Susceptibility testing profile of K. pneumoniae **strains among different clinical specimens and age groups**.

		ciprofloxacin				chloramphenicol				gentamicin				imipenem				ceftazidime	
		sensitive		resistant		sensitive		resistant		sensitive		resistant		sensitive		resistant		sensitive	
Sex	male	20	(48%)	13	(72%)	17	(46%)	16	(70%)	17	(47%)	16	(67%)	31	(56%)	2	(40%)	10	(56%)
N = 60	female	22	(52%)	5	(28%)	20	(54%)	7	(30%)	19	(53%)	8	(33%)	24	(44%)	3	(60%)	8	(44%)
p		0.082				0.076				0.143				0.49				0.95	
Sample	urine	27	(64%)	10	(56%)	24	(65%)	13	(57%)	24	(67%)	13	(54%)	37	(67%)	0	(0%)	15	(83%)
N = 60	blood	8	(19%)	6	(33%)	7	(19%)	7	(30%)	6	(17%)	8	(33%)	10	(18%)	4	(80%)	0	(0%)
	wound swab	3	(7%)	2	(11%)	3	(8%)	2	(9%)	3	(8%)	2	(8%)	4	(7%)	1	(20%)	1	(6%)
	sputum	4	(10%)	0	(0%)	3	(8%)	1	(4%)	3	(8%)	1	(4%)	4	(7%)	0	(0%)	2	(11%)
p		0.80				0.95				0.83				0.23				0.38	
Total		42		18		37		23		36		24		55		5		18	

P = *p*-value, N = number,* =significant

Table 3
The association between *K. pneumoniae* virulence genes production with sample type and mucus production

		entB				***rmpA***				***k2***				***kfu***				***magA***			
	Sample	positive		negative		positive		negative		positive		negative		positive		negative		positive		negative	
	Urine	34	(61%)	3	(75%)	2	(67%)	35	(61%)	21	(68%)	16	(55%)	24	(67%)	13	(54%)	6	(75%)	31	
	Blood	14	(25%)	0	(0%)	0	(0%)	14	(25%)	5	(16%)	9	(31%)	6	(17%)	8	(33%)	2	(25%)	12	
	Wound swab	5	(9%)	0	(0%)	0	(0%)	5	(9%)	2	(6%)	3	(10%)	4	(11%)	1	(4%)	0	(0%)	5	
	Sputum	3	(5%)	1	(25%)	1	(33%)	3	(5%)	3	(10%)	1	(3%)	2	(6%)	2	(8%)	0	(0%)	4	
p		0.73				0.43				0.86				0.86				0.24			
M	Hyper	9	(16%)	1	(25%)	0	(0%)	10	(18%)	8	(26%)	2	(7%)	4	(11%)	6	(25%)	1	(13%)	9	
	Classic	47	(84%)	3	(75%)	3	(100%)	47	(82%)	23	(74%)	27	(93%)	32	(89%)	18	(75%)	7	(88%)	43	
p		0.64				0.42				0.050				0.15				0.73			
Total		56		4		3		57		31		29		36		24		8		5	

P = *p*-value, M = Mucoviscosity

Table 4
The association between imipenem susceptibility testing, mucoviscosity, and carbapenems resistant genes

		IMP				***OXA-48***				***KPC***				***NDM***			
		Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
Imipenem	S	2	(4%)	53	(96%)	38	(69%)	17	(31%)	5	(9%)	50	(91%)	6	(11%)	49	(89%)
	R	0	(0%)	5	(100%)	3	(60%)	2	(40%)	0	(0%)	5	(100%)	0	(0%)	5	(100%)
p		0.76				0.57				0.03*				0.63			
Mucoviscosity	H	0	(0%)	10	(100%)	8	(80%)	2	(20%)	0	(0%)	10	(100%)	2	(20%)	8	(80%)
	C	2	(4%)	48	(96%)	33	(66%)	17	(34%)	5	(10%)	45	(90%)	4	(8%)	46	(92%)
p		0.52				0.39				0.30				0.26			
Total		2		58		41		19		5		55		6		48	

Abbreviations: P = p-value, R = Resistant, and S = Sensitive, H = Hypermucoid, C = Classic

Table 5
The association between *K. pneumoniae* virulence and carbapenems resistant genes production

		IMP				*OXA-48*				*KPC*				*NDM*			
		positive		negative		positive		negative		positive		negative		positive		negative	
mrkD	positive	1	(2)	46	(98%)	32	(68%)	15	(32%)	4	(9%)	43	(91%)	4	(9%)	43	(91%)
	negative	1	(8%)	12	(92%)	9	(69%)	4	(31%)	1	(8%)	12	(92%)	2	(15%)	11	(85%)
p		0.33				0.93				0.92				0.47			
entB	positive	2	(4%)	54	(96%)	39	(70%)	17	(30%)	5	(9%)	51	(91%)	4	(7%)	52	(93%)
	negative	0	(0%)	4	(100%)	2	(50%)	2	(50%)	0	(0%)	4	(100%)	2	(50%)	2	(50%)
p		0.70				0.42				0.51				0.005			
rmpA	positive	0	(0%)	3	(100%)	1	(33%)	2	(67%)	0	(0%)	3	(100%)	0	(0%)	3	(100%)
	negative	2	(4%)	55	(96%)	40	(70%)	17	(30%)	5	(9%)	52	(91%)	6	(11%)	51	(89%)
p		0.74				0.18				0.59				0.51			
k2	positive	1	(3%)	30	(97%)	21	(68%)	10	(32%)	1	(3%)	30	(97%)	3	(10%)	28	(90%)
	negative	1	(3%)	28	(97%)	20	(69%)	9	(31%)	4	(14%)	25	(86%)	3	(10%)	26	(90%)
p		0.94				0.92				0.14				0.93			
kfu	positive	0	(0%)	36	(100%)	26	(72%)	10	(28%)	1	(3%)	35	(97%)	3	(8%)	33	(92%)
	negative	2	(8%)	22	(92%)	15	(63%)	9	(38%)	4	(17%)	20	(83%)	3	(13%)	21	(88%)
p		0.08				0.43				0.05				0.60			
magA	positive	1	(13%)	7	(88%)	7	(88%)	1	(13%)	1	(13%)	7	(88%)	1	(13%)	7	(88%)
	negative	1	(2%)	51	(98%)	34	(65%)	18	(35%)	4	(8%)	48	(92%)	5	(10%)	47	(90%)
p		0.12				0.21				0.65				0.80			

Figures

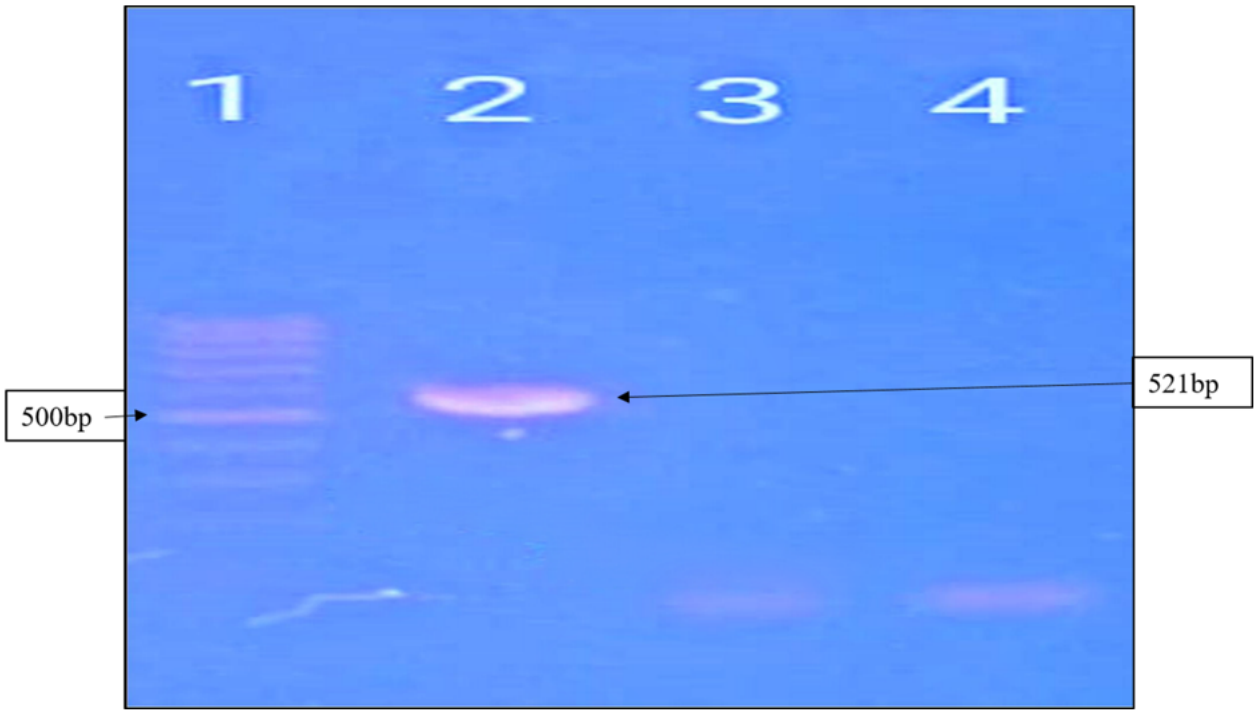

Figure 1

Amplified DNA of blaNDM gene. Lane 1, DNA ladder 100-1500bp; Lane 2, typical band size of 521bp corresponding to the molecular size of blaNDM gene; Lane 3 and 4, negative samples.

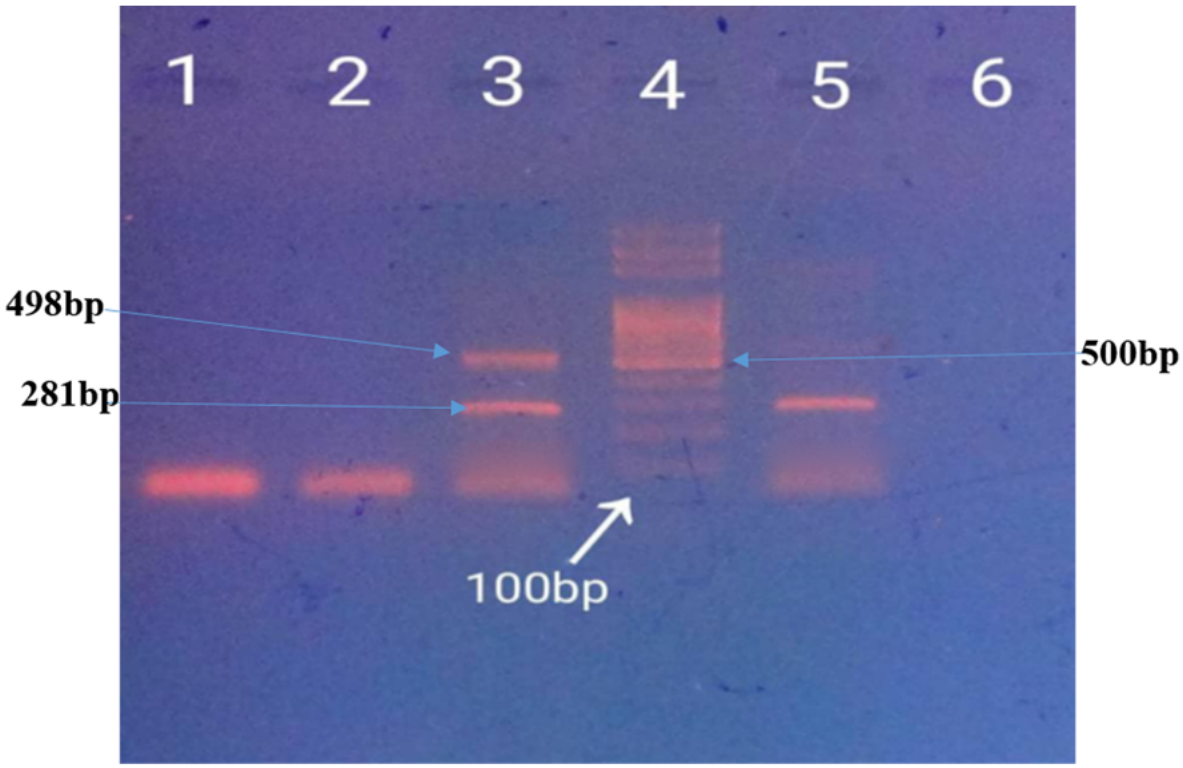

Figure 2

Amplified DNA of blaKPC and blaOXA-48 genes. Lane 4, DNA ladder 100-1500bp; Lane 3, typical bands size of 498bp and 281bp corresponding to the molecular size of blaKPC and blaOXA-48 genes respectively; Lane 5, typical band size of 281bp corresponding to the molecular size of blaOXA-48 gene; Lanes 1,2, and 6 are negative samples.

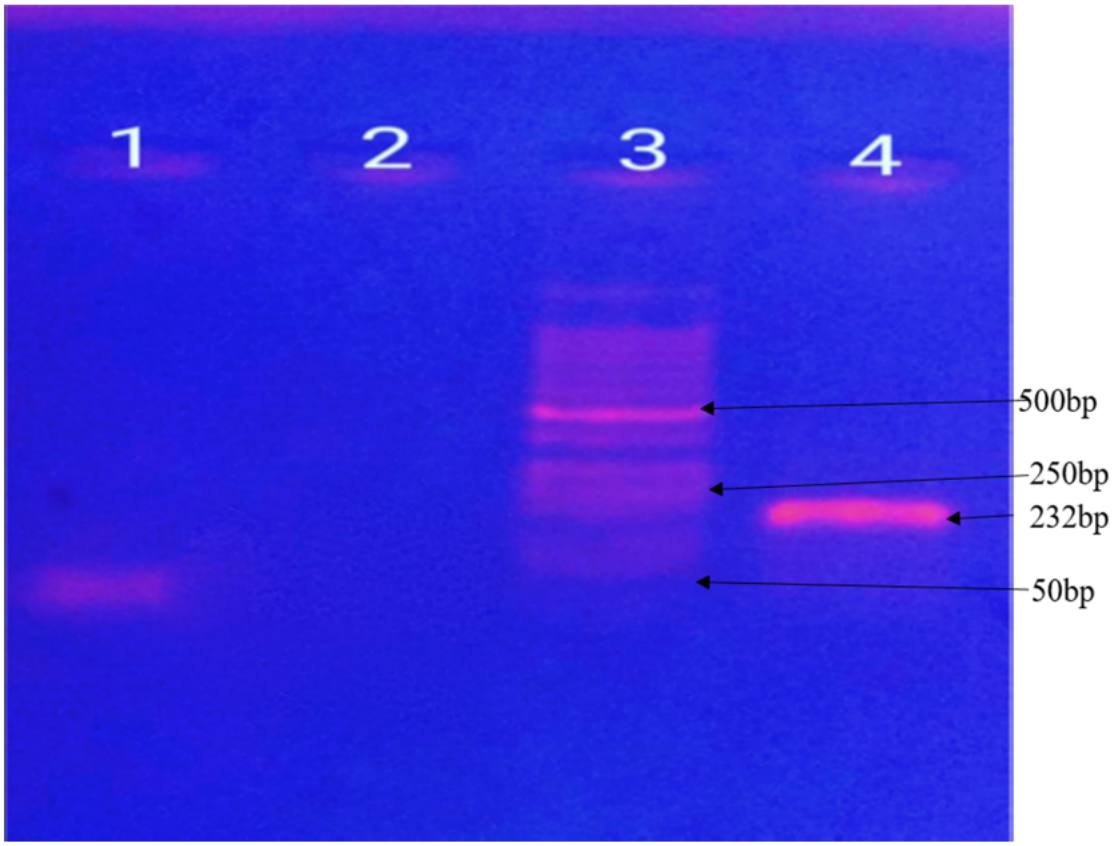

Figure 3

Amplified DNA of blaIMP gene. Lane 3, DNA ladder 50bp; Lane 4, positive sample corresponding to the molecular size of blaIMP gene; Lanes 1 and 2 are negative samples.

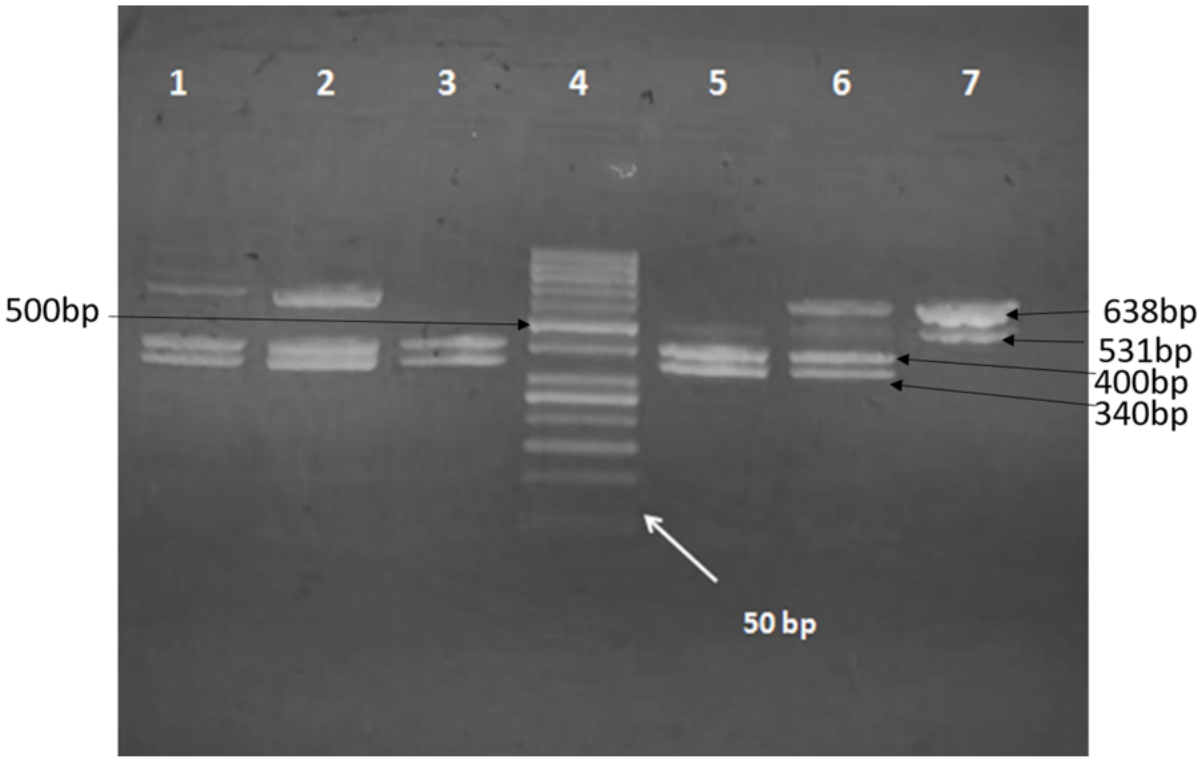

Figure 4

Multiplex PCR for amplification of K. pneumoniae virulence genes on 1.5% agarose gel electrophoresis. Lane 3 marker: 50 – 1000bp fragments. Lane 1 positive sample contain mrkD (340bp), entB (400bp) and kfu (638bp) genes. Lane 2 positive sample for mrkD and entB genes. Lane 4 positive sample for mrkD, entB, and K2 (531bp) genes. Lane 5 positive sample for mrkD, entB, K2 and kfu genes. Lane 6 positive sample for K2 and kfu genes.