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RESEARCH ARTICLE

Study of Various Virulence Genes, Biofilm Formation and Extended-Spectrum β -lactamase Resistance in *Klebsiella pneumoniae* Isolated from Urinary Tract Infections

Sanaa S. Hamam², Reem M. El Kholy³ and Maysaa E. S. Zaki^{1,*}

¹Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

²Medical Microbiology & Immunology, Faculty of Medicine, Menoufia University, Menoufia, Egypt

³Clinical Pathology, Faculty of Medicine, Menoufia University- Menoufia, Egypt

Abstract:

Objective:

The aims of the current study were to evaluate the capacity of *K. pneumoniae* isolated from hospital-acquired urinary tract infection to form biofilm, the relation of this capacity to various virulence genes and the prevalence of Extended Spectrum β -lactamases (ESBL) among these isolates by phenotypic and genotypic methods.

Material and Methods:

The study included 100 non-duplicate strains of *K. pneumoniae* isolated from 100 different urine samples from patients with hospital-acquired urinary tract infection. The isolated strains were studied for biofilm formation, ESBL production by phenotypic methods. Molecular studies were applied for the detection of ESBLs genes *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and for detection of virulence genes *fimH*, *uge*, *rmpA*, *mag A*, *wzy*, *kfa* and aerobactin genes.

Result:

The majority of the isolates had the capacity to form a biofilm (81%), with ESBL prevalence rate 41%. The most prevalent gene among ESBL producing *K. pneumoniae* was *bla*_{CTX-M} (73.2%) followed by *bla*_{SHV} (53.6%) and *bla*_{TEM} (51.2%). Among the virulence genes studied in *K. pneumoniae* isolates, the most prevalent gene was *fimH* (76%), *uge* (70%). There was significant association between ESBL production, and resistance to amikacin, cefepime, ceftazidime, gentamicin, imipenem and meropenem and biofilm production in *K. pneumoniae* isolates. There was significant association between *bla*_{CTX-M}, *bla*_{SHV}, *fimH*, *mag*, *kfa*, *wzy*, *rmpA* and aerobactin and biofilm production in *K. pneumoniae*.

Conclusion:

The present study highlights the prevalence of virulence genes among biofilm-forming strains of *K. pneumoniae* isolated from hospital-acquired urinary tract infection. Moreover, there was association between biofilm formation and ESBL production. Further studies are required to elucidate the clinical impact of the association of these different mechanisms.

Keywords: ESBL, Biofilm, *K. pneumoniae*, PCR, Virulence genes, Phenotypic methods.

Article History

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1. INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*), as a gram-negative bacillus, is known to be associated with various infections such as Urinary Tract Infection (UTI) especially hospital-acquired Catheter-Associated Urinary Tract Infection (CAUTI), sepsis and pneumonia [1, 2].

There are many virulence factors in *K. pneumoniae* associated with its pathogenicity. Among these factors is the capacity of *K. pneumoniae* to form a biofilm that protects it from the host immune response as well as from the antibiotics [3, 4]. The formation of biofilm leads to the prolonged persistence of *K. pneumoniae* on the epithelium and on the medical devices with difficulty in its eradication. There are genes related to biofilm formation in *K. pneumoniae* such as capsular genes like *rmpA* gene associated with K1/K2 virulent

* Address correspondence to this author at the Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt; Tel: 0020502258877; E-mail: May_s65@hotmail.com

type, fimbriae genes such as *fimH* and *mrkD* that regulate fimbrial types 1 and 3, respectively and genes encoding the enzymes used for the biosynthesis of lipopolysaccharide such as *uge* gene that encodes for the uridine diphosphate galacturonate 4-epimerase [5, 6] and genes that regulate iron uptake by *K. pneumoniae* including aerobactin and *kfu* genes [7]. Moreover, quorum sensing system especially type 2 and *pga* ABCD operon that regulates the synthesis and translocation of poly-b-1,6-N-acetyl-D-glucosamine adhesin are involved in the biofilm formation through enhancement of cell to cell communication and intercellular adhesion process [8].

Beside biofilm formation, clinical isolates of *K. pneumoniae* are known to possess resistance to third generation of cephalosporines through the production of Extended Spectrum β -Lactamase (ESBL).

Extended-spectrum beta-lactamase is associated with the mutation of genes that regulate β -lactamases mainly *bla_{SHV}*, *bla_{TEM}*, and *bla_{CTX-M}* genes. More than 300 different ESBLs variants have been described [9].

There are few reports about the association of biofilm and ESBL within isolates of *K. pneumoniae* from hospital-acquired urinary tract infection.

Therefore, the present study aimed to study the capacity of *K. pneumoniae* isolated from hospital-acquired urinary tract infection to form biofilm and to correlate this capacity to various virulence genes and finally detect the prevalence of ESBL among these isolates by phenotypic and genotypic methods.

2. MATERIALS AND METHODS

The study included 100 non-duplicate strains of *K. pneumoniae* isolated from 100 patients with hospital-acquired urinary tract infections from Mansoura University Hospital, Egypt as defined by CDC in a period from December 2016 till January 2018. The study was approved by Mansoura Faculty of Medicine ethical committee and approval was obtained from each participant.

2.1. Microbiological Culture

Urine samples from catheterized patients were collected as part of the routine microbiological investigation, after disinfection of the catheter collection part using 70% ethyl alcohol solution discarding the first few drops of urine and collecting 5-10 ml urine using a sterile syringe. Urine samples were immediately sent to the laboratory to be processed and examined within 2 hours. Quantitative urine culture was performed by the use of sterile calibrated loop with a culture of 1 micron of the urine onto MacConkey agar plate and incubation was performed at 37°C for 24-48 hours. The colonies were identified by gram staining and biochemical identification was performed by VITEK 2 system according to the instructions of the manufacturers. The isolated *K. pneumoniae* were subjected to antibiotics susceptibility testing by disc diffusion method, determination of ESBL done by combined disc diffusion method and study of biofilm formation

by Microtiter plate method. Molecular studies were carried out for the detection of virulence genes and *bla* genes.

2.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility was performed by discs diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI 2018) [10]. The used discs were: amikacin, cefotaxime, ceftazidime, cefepime, ciprofloxacin, levofloxacin, gentamicin, piperacillin-tazobactam, trimethoprim/sulfamethoxazole, imipenem, and meropenem (Oxoid-UK). The interpretation of the results was done according to CLSI 2018 [10].

2.3. ESBL Production

Determination of ESBL by Combined disk diffusion confirmatory test.

Klebsiella pneumoniae strains resistant to ceftazidime (30 μ g) and/ or cefotaxime (30 μ g) were subjected to the screening of ESBL enzymes production by combined disk diffusion confirmatory test. The principle of the test depends upon the use of ceftazidime and cefotaxime discs alone and conjugated with clavulanic acid. The increase of the inhibition zone diameter around discs conjugated with clavulanic acid ≥ 5 mm was interpreted as positive ESBL production.

The suspension of overnight growth of *K. pneumoniae* in Muller-Hinton broth was prepared with adjusted turbidity to 0.5 McFarland and was subculture on Muller-Hinton agar medium and antibiotic discs containing ceftazidime, ceftazidime (30 μ g)+clavulanic acid (10 μ g), cefotaxime, and cefotaxime (30 μ g + clavulanic (10 μ g) pairs were placed with 20 mm space between them. Plates were incubated at 37°C for 24 hours. The increase of growth inhibition zone ≥ 5 mm around ceftazidime/clavulanic acid and cefotaxime/clavulanic compared to ceftazidime and cefotaxime respectively was regarded as an ESBL producing isolate [11]. The strain of *Klebsiella pneumoniae* (ATCC 700603) was used as a positive quality control strain for ESBL production.

2.4. Biofilm Assay

Biofilm formation by *K. pneumoniae* isolates was assessed by the semi-quantitative method [12].

Briefly, 0.5 McFarland suspensions of *K. pneumoniae* isolates were prepared using brain heart infusion broth (Oxoid-UK) and 100 microliters of the suspensions were inoculated onto wells of 96-well polystyrene plate and incubated at 37°C for 18 hours. Negative control well was inoculated with sterile brain heart infusion broth. Then, wells were stained with 200 microliters of crystal violet with concentration 0.5% for 20 minutes and the excess stain was washed by 200 microns sterile distilled water for three times. Then the stain was eluted with 200 microliters of 95% of ethanol and the optical density was measured at 570 nm by enzyme-linked immunoassay reader. The positive Optical Density (OD) was defined as three standard deviations above the mean OD of the negative control. The *K. pneumoniae* NTUH-K2044 strain was used as positive control.

2.5. Molecular Studies of *K. pneumoniae*

2.5.1. Extraction of DNA

DNA of the isolated *K. pneumoniae* was prepared by boiling method. Briefly, colonies were suspended in 100 microliters of sterile distilled water and boiled at 100°C in the water bath for 15 minutes then rapidly cooled at -20°C for one hour, then centrifugation and the supernatant were preserved for the use in the amplification processes [13].

2.5.2. PCR for Virulence Genes of *K. pneumoniae*

PCR was performed for the detection of virulence genes for *K. pneumoniae*. The genes detected and the sequences of the used primers were summarized in Table 1. The conditions of PCR were performed according to Shah *et al.*, 2017 [13] for each gene. Negative controls were included as sterile distilled water and *K. pneumoniae* ATCC13883 was used as positive control. The products of the amplifications were analyzed by the use of gel electrophoresis 1.5% stained with ethidium bromide and the bands were visualized by UV.

2.5.3. Multiplex PCR for ESBL Genes

The extracted DNA was used for multiplex PCR for the detection of ESBL genes *bla_{TEM}*, *bla_{SHV}* and *bla_{CTX-M}*. The sequences of the used primers were shown in Table 1. The amplification steps were primary denaturation at 94°C for 4

minutes, then 35 cycles of amplification (94°C for 45 seconds, 55°C for 45 seconds and 72°C for 1 minute) with final extension at 72°C for 7 minutes. *K. pneumoniae* ATCC 7881 was used as a positive control. The products of the amplifications were analyzed by the use of gel electrophoresis 1.5% stained with ethidium bromide and the bands were visualized by UV [13].

2.5.4. Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (SPSS) version 24. The quantitative data were presented as mean, standard deviations and ranges and qualitative data was presented by number and percentages. The comparison between qualitative data was done by the use of chi-square. P-value was considered significant if it was <0.05.

3. RESULTS

The study included non-duplicate 100 isolates of *K. pneumoniae*. The majority of the isolates had the capacity to form biofilm (81 isolates, 81%) with ESBL prevalence rate 41% (41 isolates). Forty-one isolates had the combined capacity to form biofilm and were ESBL, 40 isolates had the capacity to form biofilm and were non ESBL producers and 19 isolates were non ESBL and lack the capacity to form biofilm (Fig. 1).

Table 1. Genes, sequences of the used primers and the bp of the used products.

Gene	Sequence	bp	Positive Control
<i>FimH-1</i>	F: 5'-ATG AAC GCC TGG TCC TTT GC-3 R: 5'-GCT GAA CGC CTA TCC CCT GC-3	688	<i>E. coli</i> 700336
<i>mrkD</i>	F: 5'-CCA CCA ACT ATT CCC TCG AA -3 R: 5'-ATG GAA CCC ACA TCG ACA TT-3	240	<i>K. pneumoniae</i> ATCC BAA-2473
<i>magA</i> (K1)	F: 5'-GGT GCT CTT TAC ATC ATT GC-3 R: 5'-GCA ATG GCC ATT TGC GTT AG-3	1282	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> Strain K6 ATCC 700603
<i>wzy</i> (K2)	F: 5'-GAC CCG ATA TTC ATA CTT GAC AGA G-3 R: 5'-CCT GAA GTA AAA TCG TAA ATA GAT GGC-3	641	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> Strain K6 [ATCC® 700603™]
<i>rmpA</i>	F: 5'-ACT GGG CTA CCT CTG CTT CA -3 R: 5'-CTT GCA TGA GCC ATC TTT CA-3	535	<i>Klebsiella pneumoniae</i> 1100770
<i>uge</i>	F: 5'-TCT TCA CGC CTT CCT TCA CT-3 R: 5'-GAT CAT CCG GTC TCC CTG TA-3	534	<i>K. pneumoniae</i> ATCC BAA2473
<i>kfu</i>	F: 5'-GAA GTG ACG CTG TTT CTG GC -3 R: 5'-TTT CGT GTG GCC AGT GAC TC-3	797	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> (Schroeter) Trevisan ATCC13882
Aerobactin	F: 5'-GCA TAG GCG GAT ACG AAC AT-3 R: 5'-CAC AGG GCA ATT GCT TAC C T-3	556	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> Strain K6 [ATCC 700603
<i>bla_{TEM}</i>	F: 5'-ATA AAA TTC TTG AAG ACG AAA -3 R: 5'-GAC AGT TAC CAA TGC TTA ATC -3	1080	<i>K. pneumoniae</i> ATCC 7881
<i>bla_{SHV}</i>	F: 5'-GGG TTA TTC TTA TTT GTC GC -3 R: 5'-TTA GCG TTG CCA GTG CTC-3	930	<i>K. pneumoniae</i> ATCC 7881
<i>bla_{CTX-M}</i>	F: 5'-GCT ATG TGC AGT ACC AGT AA-3 R: 5'-ACC AGA ATG AGC GGC GC-3	585	<i>K. pneumoniae</i> ATCC 7881

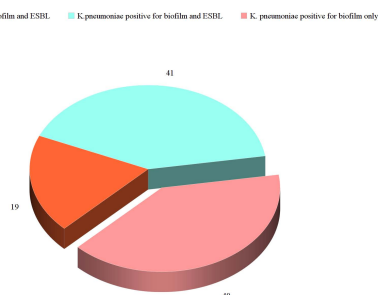


Fig (1). The frequency of ESBLs production and biofilm formation among isolated *K. pneumoniae*.

Fig (2). shows the optical density of biofilm formed by each isolate. The genes detected among ESBL producing *K. pneumoniae* was *bla_{CTX-M}* in 30 isolates followed by *bla_{SHV}* 22 isolates and *bla_{TEM}* in 21 isolates. Multiple genes were present in 25 of the isolates. Among the virulent genes' studies in *K. pneumoniae* isolates, the most prevalent gene was *fimH* (76%), *uge* (70%), *rmpA* (62%), *mag* (59%), *wzy* (59%) and aerobactin gene (51%). Lower prevalence was detected for and

kfa gene (33%).

There was significant association between ESBL production, and resistance to amikacin, cefepime, ceftazidime, gentamicin, imipenem and meropenem and biofilm-producing *K. pneumoniae* data not shown. There was a significant association between *bla_{CTX-M}*, *bla_{SHV}*, *fimH*, *magA*, *kfa*, *wzy*, *rmpA* and aerobactin and biofilm-producing *K. pneumoniae* (Table 2).

Table 2. Comparison between biofilm producing and non-biofilm producing *K. pneumoniae* species regarding genes associated with ESBLs production and other virulence genes.

ESBLs and Other Virulence Genes	Biofilm forming <i>K. pneumoniae</i> (n=81) No. (%)	Non-Biofilm forming <i>K. pneumoniae</i> (n=29) No. (%)	P
<i>bla_{TEM}</i>	12 (14.8%)	9 (31%)	P=0.007
<i>bla_{SHV}</i>	11 (13.6%)	11 (37.9%)	P=0.005
<i>bla_{CTX-M}</i>	30 (37%)	0 (0%)	P=0.0002
<i>fimH</i>	72 (88.9%)	4 (13.8%)	P=0.0001
<i>magA</i>	49 (60.5%)	10 (34.4%)	P=0.0001
<i>wzy</i>	49(60.5%)	10(34.4%)	P=0.0001
<i>rmpA</i>	58 (71.6%)	4 (13.8%)	P=0.0001
<i>uge</i>	59 (72.8%)	11 (37.9%)	P=0.2
<i>kfa</i>	33 (40.7%)	0 (0%)	P=0.0001
aerobact	48 (59.3%)	3 (10.3%)	P=0.0001

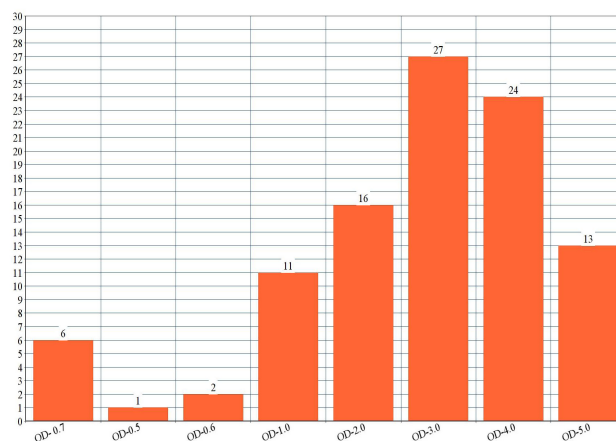


Fig. (2). Distribution of OD of biofilm formation among isolated *K. pneumoniae*.

4. DISCUSSION

Klebsiella pneumoniae has emerged as an important pathogen causing around 20% of infections in the hospitals worldwide [14].

There are several factors that increase the virulence of *K. pneumoniae*, of which the capacity to form biofilm is prevalent. The majority of the isolates in the present study had the capacity to form biofilm (81%). This finding was similar to the previous report concerning isolates from urinary tract infections [15]. The formation of biofilm protects *K. pneumoniae* species against the host immune responses, the action of antibiotics and enhance its persistence [16, 17].

One of the other factors associated with infection by *K. pneumoniae* is the emergence of resistance to antibiotics by different mechanisms. The common mechanism is ESBL production. In the present study, the prevalence of ESBL production was 41%. The prevalence of ESBL production among *K. pneumoniae* species varied from 30% up to 65% in different studies [18, 19]. The variations of the prevalence of ESBL depend upon the empirical use of antibiotics in local regions with positive pressure on *K. pneumoniae* leading to the selection of resistant strains.

The most prevalent genes among ESBL producing *K. pneumoniae* were *bla*_{CTX-M} followed by *bla*_{SHV} and *bla*_{TEM}. Several reports had a similar finding with *bla*_{CTX-M} was the most prevalent gene followed by *bla*_{TEM} and *bla*_{SHV} [20, 21]. The high prevalence of *bla*_{CTX-M} among *K. pneumoniae* may represent selective pressure due to the wide prescriptions of cephalosporins, particularly cefotaxime and ceftriaxone in many geographical regions including Egypt [22]. However, the prevalence rates vary not only between different countries but even between different hospitals in the same country [23].

The ESBL enzymes are encoded by plasmid genes, therefore, it is expected for one isolate to carry different genes as the mixed genes were present in 65.8% of the isolates in our study. Similar findings were reported by [20]. Further studies are required to understand the mechanisms mediated by multiple *bla* genes on the same isolate.

The present study revealed that the most prevalent virulence genes among *K. pneumoniae* species were *fimH* (76%), *uge* (70%), *rmpA* (62%), *mag* (59%) and *wzy* (59%) and the lowest prevalence was detected for aerobactin gene (50%) and *kfa* gene (33%). Similar prevalence rates were reported for *fimH*, *uge* and *rmpA* genes [23, 24]. The prevalence of aerobactin gene varies in different studies from 3% up to 92.8% [25, 26]. The difference in the prevalence of virulence genes appears to be associated with the difference of the clinical sources of the isolated *K. pneumoniae* species.

Several studies demonstrated an association between the aerobactin gene and the regulatory genes of mucous in *K. pneumoniae* [26, 27]. There are cumulative data that the virulence of *K. pneumoniae* is enhanced by the presence of aerobactin due to the presence of other multiple virulence genes on the same plasmid carrying it [27].

There was a significant association between resistance to amikacin, cefepime, ceftazidime, gentamicin, imipenem and meropenem and biofilm production by *K. pneumoniae*. The finding was explained by several mechanisms such as the adherent bacterial cells are resistant to the actions of different antibiotics due to the limitation of the penetration of the antibiotics, beside the slow growth of the bacterial cells inside the biofilm [16]. Moreover, the exchange of plasmids that carry antibiotics resistance genes between contact microbial cells may play role [28, 29]. ESBL production by isolated *K. pneumoniae* species was significantly associated with biofilm formation. This finding was similar to previous reports [29, 30]. Again, this finding can be explained by the formation of protective environment to *K. pneumoniae* away from the antibiotics and the transmission of resistant genes. This was confirmed in the present study by significant association of *bla*_{SHV} and *bla*_{CTX-M} genes with biofilm formation.

Virulence genes of *K. pneumoniae*, *fimH*, *magA*, *kfa*, *rmpA* and aerobactin had a significant association with biofilm formation. Several studies correlated the association of genes of fimbriae, capsule and lipopolysaccharides with the biofilm formation by *K. pneumoniae* through initial adhesion by fimbriae and lipopolysaccharide followed by coverage of substratum and construction of mature biofilm architecture by the capsule [31, 32].

CONCLUSION

The present study highlights the prevalence of virulence genes among biofilm-forming strains of *K. pneumoniae* isolated from hospital-acquired urinary tract infections. Moreover, there was an association between biofilm formation and ESBL production. Further studies are required to elucidate the clinical impact of the association of these different mechanisms.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by Mansoura Faculty of Medicine ethical committee, Egypt.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all the participants prior to publication.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none

REFERENCES

- [1] Maria SA, Paolo O, Donatella P, Fernanda P, Luisa CM. An overview of carbapenem-resistant *Klebsiella pneumoniae*: Epidemiology and control measures. *Rev Med Microbiol* 2014; 25: 7-14. [http://dx.doi.org/10.1097/MRM.0b013e328365c51e]
- [2] Murphy CN, Clegg S. *Klebsiella pneumoniae* and type 3 fimbriae: Nosocomial infection, regulation and biofilm formation. *Future Microbiol* 2012; 7(8): 991-1002. [http://dx.doi.org/10.2217/fmb.12.74] [PMID: 22913357]
- [3] Bandeira M, Carvalho PA, Duarte A, Jordao L. Exploring dangerous connections between *Klebsiella pneumoniae* biofilms and healthcare-associated infections. *Pathogens* 2014; 3(3): 720-31. [http://dx.doi.org/10.3390/pathogens3030720] [PMID: 25438020]
- [4] Chung PY. The emerging problems of *Klebsiella pneumoniae* infections: Carbapenem resistance and biofilm formation. *FEMS Microbiol Lett* 2016; 363(20):fnw219 [http://dx.doi.org/10.1093/femsle/fnw219] [PMID: 27664057]
- [5] Balestrino D, Ghigo JM, Charbonnel N, Haagenen JA, Forestier C. The characterization of functions involved in the establishment and maturation of *Klebsiella pneumoniae in vitro* biofilm reveals dual roles for surface exopolysaccharides. *Environ Microbiol* 2008; 10(3): 685-701. [http://dx.doi.org/10.1111/j.1462-2920.2007.01491.x] [PMID: 1823 7304]
- [6] Alcántar-Curiel MD, Blackburn D, Saldaña Z, et al. Multi-functional analysis of *Klebsiella pneumoniae* fimbrial types in adherence and biofilm formation. *Virulence* 2013; 4(2): 129-38. [http://dx.doi.org/10.4161/viru.22974] [PMID: 23302788]
- [7] Ma LC, Fang CT, Lee CZ, Shun CT, Wang JT. Genomic heterogeneity in *Klebsiella pneumoniae* strains is associated with primary pyogenic liver abscess and metastatic infection. *J Infect Dis* 2005; 192(1): 117-28. [http://dx.doi.org/10.1086/430619] [PMID: 15942901]
- [8] Chen KM, Chiang MK, Wang M, Ho HC, Lu MC, Lai YC. The role of pgaC in *Klebsiella pneumoniae* virulence and biofilm formation. *Microb Pathog* 2014; 77: 89-99.
- [9] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. *Clin Microbiol Rev* 2005; 18(4): 657-86. [http://dx.doi.org/10.1128/CMR.18.4.657-686.2005] [PMID: 1622 3952]
- [10] Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twentieth informational supplement 2015. http://shop.clsi.org/site/Sample_pdf/M100S25_sample.pdf
- [11] Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and Carbapenemase issues. *J Clin Microbiol* 2010; 48(4): 1019-25. [http://dx.doi.org/10.1128/JCM.00219-10] [PMID: 20181902]
- [12] 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):e23500 [http://dx.doi.org/10.1371/journal.pone.0023500] [PMID: 21858144]
- [13] Shah RK, Ni ZH, Sun XY, Wang GQ, Li F. The determination and correlation of various virulence genes, ESBL, serum bactericidal effect and biofilm formation of clinical isolated classical *klebsiella pneumoniae* and hypervirulent *klebsiella pneumoniae* from respiratory tract infected patients. *Pol J Microbiol* 2017; 66(4): 501-8. [http://dx.doi.org/10.5604/01.3001.0010.7042] [PMID: 29319515]
- [14] De Rosa FG, Corcione S, Cavallo R, Di Perri G, Bassetti M. Critical issues for *Klebsiella pneumoniae* KPC-carbapenemase producing *K. pneumoniae* infections: A critical agenda. *Future Microbiol* 2015; 10(2): 283-94. [http://dx.doi.org/10.2217/fmb.14.121] [PMID: 25689539]
- [15] Vuotto C, Longo F, Pascolini C, et al. Biofilm formation and antibiotic resistance in *Klebsiella pneumoniae* urinary strains. *J Appl Microbiol* 2017; 123(4): 1003-18. [http://dx.doi.org/10.1111/jam.13553] [PMID: 28731269]
- [16] Vuotto C, Longo F, Balice MP, Donelli G, Varaldo PE. Antibiotic resistance related to biofilm formation in *Klebsiella pneumoniae*. *Pathogens* 2014; 3(3): 743-58. [http://dx.doi.org/10.3390/pathogens3030743] [PMID: 25438022]
- [17] Hawkey PM. Prevalence and clonality of extended-spectrum beta-lactamases in Asia. *Clin Microbiol Infect* 2008; 14(Suppl. 1): 159-65. [http://dx.doi.org/10.1111/j.1469-0691.2007.01855.x] [PMID: 1815 4540]
- [18] Patzer JA, Dzierzanowska D, Pawińska A, Turner PJ. High activity of meropenem against Gram-negative bacteria from a paediatric Intensive Care Unit, 2001-2005. *Int J Antimicrob Agents* 2007; 29(3): 285-8. [http://dx.doi.org/10.1016/j.ijantimicag.2006.09.012] [PMID: 17257 814]
- [19] Patzer JA, Dzierzanowska D. Increase of imipenem resistance among *Pseudomonas aeruginosa* isolates from a Polish paediatric hospital (1993-2002). *Int J Antimicrob Agents* 2007; 29(2): 153-8. [http://dx.doi.org/10.1016/j.ijantimicag.2006.08.044] [PMID: 1715 7481]
- [20] Mahdi Yahya Mohsen S, Hamzah HA, Muhammad Imad Al-Deen M, Baharudin R. Antimicrobial Susceptibility of *Klebsiella pneumoniae* and *Escherichia coli* with Extended-Spectrum β -lactamase associated Genes in Hospital Tengku Ampuan Afzan, Kuantan, Pahang. *Malays J Med Sci* 2016; 23(2): 14-20. [PMID: 27547110]
- [21] Al-Marzooq F, Mohd Yusof MY, Tay ST. Molecular analysis of antibiotic resistance determinants and Plasmids in Malaysian isolates of multidrug resistant *Klebsiella pneumoniae*. *PLoS One* 2015; 10(7):e0133654 [http://dx.doi.org/10.1371/journal.pone.0133654] [PMID: 26203651]
- [22] Ahmed O, El-Hady SA, Ahmed TM, Ahmed IZ. Detection of *bla_{SHV}* and *bla_{CTX-M}* genes in ESBL producing *Klebsiella pneumoniae* isolated from Egyptian patients with suspected nosocomial infections. *Egypt J of Med Human Gen* 2013; 14(3): 277-83.
- [23] Guo Y, Wang S, Zhan L, et al. Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. *Front Cell Infect Microbiol* 2017; 7: 24-9. [http://dx.doi.org/10.3389/fcimb.2017.00024] [PMID: 28203549]
- [24] Candan ED, Aksöz N. *Klebsiella pneumoniae*: Characteristics of carbapenem resistance and virulence factors. *Acta Biochim Pol* 2015; 62(4): 867-74. [http://dx.doi.org/10.18388/abp.2015_1148] [PMID: 26637376]
- [25] Alizade H, Jajarmi M, Aflatoonian M, Neyestanaki DK, Shoja S, Ghanbarpour R. Comparative prevalence of *bla_{CTX-M}* gene with virulence genes and serotypes in *Klebsiella pneumoniae*. *Jund J Microb* 2018; 11(4):e61285
- [26] Russo TA, Shon AS, Bean JM, et al. Hypervirulent *K. pneumoniae* secretes more and more active iron-acquisition molecules than "classical" *K. pneumoniae* thereby enhancing its virulence. *PLoS One* 2011; 6(10):e26734 [http://dx.doi.org/10.1371/journal.pone.0026734] [PMID: 22039542]
- [27] Jung SW, Chae HJ, Park YJ, et al. Microbiological and clinical characteristics of bacteraemia caused by the hypermucoviscosity phenotype of *Klebsiella pneumoniae* in Korea. *Epidemiol Infect* 2013; 141(2): 334-40. [http://dx.doi.org/10.1017/S0950268812000933] [PMID: 22578630]
- [28] Angel Díaz M, Ramón Hernández J, Martínez-Martínez L, Rodríguez-Baño J, Pascual A. Grupo de Estudio de Infección Hospitalaria (GEIH). [Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Spanish hospitals: 2nd multicenter study (GEIH-BLEE project, 2006)]. *Enferm Infecc Microbiol Clin* 2009; 27(9): 503-10. [http://dx.doi.org/10.1016/j.eimc.2008.09.006] [PMID: 19329230]
- [29] Juhás M. Horizontal gene transfer in human pathogens. *Crit Rev Microbiol* 2015; 41(1): 101-8. [http://dx.doi.org/10.3109/1040841X.2013.804031] [PMID: 2386 2575]
- [30] Singhai M, Malik A, Shahid M, Malik MA, Goyal R. A study on device-related infections with special reference to biofilm production and antibiotic resistance. *J Glob Infect Dis* 2012; 4(4): 193-8. [http://dx.doi.org/10.4103/0974-777X.103896] [PMID: 23326076]
- [31] Nepal HP, Neopane P, Shrestha R, et al. Biofilm formation and

antimicrobial resistance in *Klebsiella pneumoniae* isolated from patients visiting a tertiary care center of Nepal. Asian Pac J Trop Dis 2017; 7(6): 347-50.
[<http://dx.doi.org/10.12980/apitd.7.2017D7-15>]

[32] Johnson JG, Clegg S. Role of MrkJ, a phosphodiesterase, in type 3 fimbrial expression and biofilm formation in *Klebsiella pneumoniae*. J Bacteriol 2010; 192(15): 3944-50.
[<http://dx.doi.org/10.1128/JB.00304-10>] [PMID: 20511505]

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