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Phenotypic and Genotypic Study of Some Virulence Factors for *Klebsiella Pneumonia* Isolation from Diabetic Foot Ulcer and Urinary Tract Infection

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Abstract
The pathogenicity of *K. pneumoniae* bacteria is associated with several virulence factors that allow it to evade the host's innate immune mechanisms. To establish infection, *K. pneumoniae* must overcome mechanical and chemical barriers and escape host humoral and cellular innate defenses. After gaining access to the host, the invading organisms are recognized by the immune cells. In this study, 42 *K. pneumoniae* clinical specimens isolated from diabetic patients whose suffering from urinary tract infection and diabetic foot ulcer. Present virulence factors of *K.pneumoniae* detect by phenotypic and genotypic methods. The *K. pneumoniae* isolates which were positive for various virulence factors were as follows on Slim layer 34(80.9%), Gelatinase 11(26.1%), Bioflim 41(97.6%), Lipase 0(0).
The genotypic detection showed that *K. pneumoniae* had virulence genes and were 8(40%) for *wab*G and 8(40%) for *fim*H.

Keywords: *Klebsiella Pneumonia*, Diabetic Foot Ulcer, Urinary Tract Infection, Virulence Factors

Introduction
K. pneumoniae is one of an opportunistic pathogen causing hospital-acquired and community-acquired infections. *K. pneumoniae* is a member of the family Enterobacteriaceae. It is a non-fastidious, Gram negative bacillus, which is usually encapsulated (Bhatia *et al.*, 2021).
K. pneumoniae grows rapidly on ordinary media and their colonies looks large pink, round, mucoid on MacConkey agar indicating fermentation of lactose and acid production (Sharma *et al*., 2015) [23], presence can be in several forms, the most important of which is soil, water, and sewage ecosystems. As for human in particular It is also found on various human body sites and organ systems, including skin, nose, throat, and intestinal tract, as part of the natural microflora (Gomez, 2017) [9].
K. pneumoniae must overcome mechanical and chemical barriers in addition to host humoral and cellular innate defenses in order to initiate infection. After invading organisms gain entrance to the host, they become recognized by immune cells via pattern recognition receptors, triggering the production of numerous immune mediators. (Happel, 2005) [12].K. pneumoniae pathogenicity is related with various virulence factors that allow it to overcome the host's innate immune defenses. K. pneumoniae includes capsules, exopolysaccharides linked to mucoviscosity, and lipopolysaccharide adhesins, and iron uptake systems. (kuy *et al*., 2017).*K. pneumoniae* cells within biofilms are partially protected from immune defenses (Jafari-Sales, *et al*., 2020), Bacterial biofilms secrete a mixture of polysaccharides, proteins, fatty acids, and a variety of nucleic acids which is referred to as extracellular polymeric substance or EPS (Gupta *et al*., 2016) [11]. Gelatinase is an enzyme that hydrolyze gelatin into gelatin liquefaction, gelatinase considerable as targets for drug development because of their potential role in connective tissue degradation associated with tumor metastasis (Nursyam *et al*., 2018) [17].

Method
Bacteria
Different enrichment, differential, and selective media were used to culture the bacteria collected from diabetic patients suffering from diabetic foot ulcer and urinary tract infection who attended to the diabetes center in AL-Sader Hospital/ Al Najaf Province. After the bacteria had grown in the medium, a biochemical test was used to identify the bacterium.

Detection of Virulence Factors

Slim layer:

Detection of Slim Layer

In Congo red agar (CRA) test, this method is described by Freeman *et al*., (1989) [7] an alternative method of screening biofilm formation by *klebsilla* isolates; which requires the use of a specially prepared solid medium -brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. A positive result was indicated by positive result black colonies with a dry crystalline consistency, intermediate result Black-smooth colonies, Negative result Red-smooth colony (Freeman *et al*., 1989) [7].

Detection Biofilm Formation by Micro Titer Plates (MTP) Method

Detection of biofilm formation by tissue culture plate method (TCP) assay according to Lizcano *et al*., (2010) [16].

Gelatinase Production:

Gelatinase production test was performed by inoculating Nutrient gelatine agar with bacterial colonise and incubated at 37 C for 1-14 days. The liquefaction media indicated the positive result (Collee *et al*, 1996) [5].

Production of Lipase:

The production of extracellular lipase enzyme was tested on trypton soya agar supplemented with 1 % tween 80, after incubation for 48 h at 37 ◦C.

Genomic DNA Extraction:

Boiling was used to process the extracted bacteria's DNA. Many bacterial colonies were placed in 300 microliters of sterile TE buffer, boiled for 15 minutes at 100 degrees Celsius in a water bath, quickly cooled to 20 degrees Celsius for 15 minutes, centrifuged, and the supernatant was saved for use in the later amplification operations (Shah *et al*., 2017) [22].

Amplification of Genes:

The following three primers were applied to amplify the genes of bacterial isolates DNA extracts, including *fim H, wac G*, the following additions were 8 µL of master mix, 5 µL of DNA template, 1.5 µL for each primer, and 4 µL of deionized water used in PCR. According to the manufacturer's instructions for Promega Biosystem, the protocol was followed. Amplification was carried out with the following thermal cycling conditions: 3 min at 95°C, 30 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C, and a final extension for 5 min at 72°C(fimH gene), 5 minutes of pre-denaturation at 95°C, followed by 30 cycles: 1 minute at 94°C, 1 minute at 58°C, 1 minute at 72°C and 10 minutes of final elongation at 72°C (wabG gene).

Table1: Primers used in this study

Primer Type	Primer sequence (5'-3')	Amplicon size (pb)	Size	Reference
wabG	F R	ACCATCGGCCATTTGATAGA CGGACTGGCAGATCCATATC	683	Ye *et al*, 2006 Yu *et al*., 2006
fimH	F R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	506	Johnson, 2000

Result and Discussion

Detection Some Virulence Factors of K. Pneumonia by Phenotypic Methods

In this study 34 (80.9%) of *K. pneumoni* isolates appeared the ability on production slim layer layer by Congo Red Agar (CRA) Assay. Our finding was disagreement with result of Panjaitan *et al*(2021) [19] and AlDoori *et al*(2020) [3] whose showed 100% *K. pneumonia* was produce slim layer by Congo Red Agar (CRA), and41 (97.6%) of *K. pneumoni* isolates was biofilm formation, this result agree with (Cerón *et al*,2022) [4] whose showed 95% from *K. pneumonia* was able on biofilm formation, and disagree with (Hullur *et al*,2022) [14] whose showed the ratio was 54%.

Fig 1: Slim layer formation by *K. pneumonia* on Congo red agar

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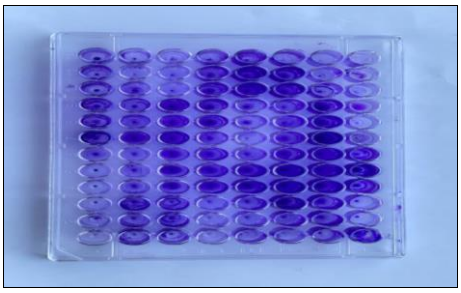

Fig 2: Biofilm formation of *K. pneumonia* on microtiter plate

The results showed that (26.1%) of *K.pneumonia* was produced gelatinase enzyme (Fig 3). This result differs with the finding of study performed by (Sekowska *et al*., 2006, Ramakrishnan *et al*., 2019) [21, 20] they have found 8.9% and 41% respectively of the isolates were positive for gelatinase production.

The results showed that all *K. pneumonia* gave negative result for lipase enzyme production. This result disagree with (Devanathan *et al.*, 2022) [6] who showed 56, 6% from *K. pneumonia* gave positive result for lipase enzyme production.

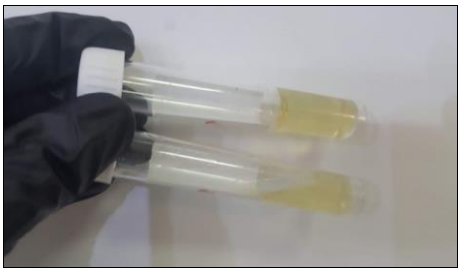

Fig 3: Ability of *K. pneumonia* on produce Gelatinase

Table 2: Virulence factor of *K.pneumonia*

Virulence Factors	Positive (%)	Negative (%)
Slim layer	34(80.9%)	8(19.1%)
Gelatinase	11(26.1%)	31(73.8)
Bioflim	41(97.6)	1(2.3%)
Lipase	0	42(100%)

Detection Some Virulence Factors of *K. Pneumonia* by Genotypic Methods

The result showed the *wabG* was detected in 8(40%) *K. pneumonia* (Fig 4). Our result agree with (Vargas *et al*., 2019) [24] who showed the wabG was 48.5% from *K.pneumoniae* isolates have *wabG* gene. And disagreement with (Guo *et al*., 2023) [10] who showed the wabG was 100% from *K.pneumoniae* isolates have *wabG* gene.

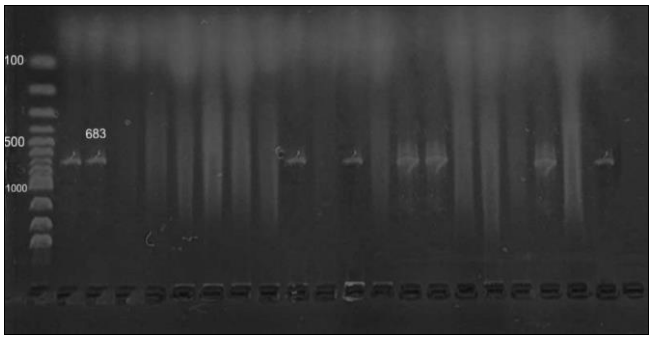

Fig 4: Determined wabG gene regions in K. pneumoniae

While fimH gen was detected 8(40%)in *K. Pneumonia wich close to* Al-Kraety *et al*.(2020) [1] that show 35% from *K.pneumoniae* isolates have *fimH* gene. And disagreement with (Hasan *et al*., 2022) [13] who showed the fim was 100% from *K.pneumoniae* isolates have *fim* gene.

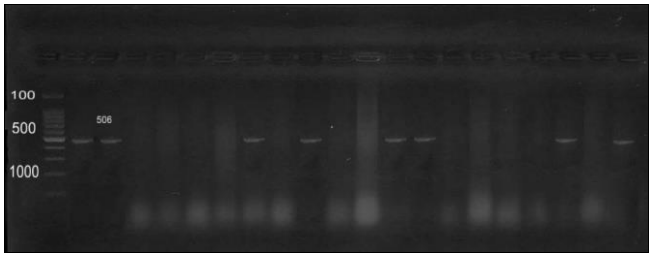

Fig 5: Determined fim H gene regions in K. pneumoniae

References

1. Al-Kraety IAA, Alquraishi ZHO, Alsadawi AA. Molecular study of fimh gene in Klebisella pneumoniae isolated from urinary catheter patients. Indian Journal of Forensic Medicine & Toxicology. 2020; 14(2):473-477.
2. Bhatia P, Sharma A, George AJ, Anvitha D, Kumar P, Dwivedi V. Correlation of drug resistance pattern with Lipase Production in against ESKAPE: An update. Heliyon. 2022; 7(2):e06310.
3. AlDoori IHA, Mahal JD, Maaroof MN. Determination of genes responsible for some virulence factors of bacteria isolated from contaminated groundwater. Eurasia J Biosci. 2020; 14:4207-4215.
4. Cerón AB, Pérez EM, Cortés LRG, Jiménez EAR, Paniagua FV, Contreras GLP. Hypervirulence and Multiresistance to Antibiotics in Klebsiella pneumoniae Strains Isolated from Patients with Hospital-and Community-Acquired Infections in a Mexican Medical Center Microorganisms. 2022; 10:p2043.
5. Collee JG, Fraser AG, Marmiom BP, Simmon A. Mackie and McCarteny Practical Medical Microbiology. 4th ed. Churchill Livingstone Inc., USA, 1996.
6. Devanathan K, Sivaraman U, Subramaniam P, Easow A. Correlation of drug resistance pattern with Lipase Production in clinical isolates of Klebsiella pneumonia. Research Square, 2022.
7. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J. Clin. Pathol. 1989; 42:872-874.
8. Ku YH, Chuang YC, Chen CC, Lee MF, Yang YC, Tang HJ, Yu WL. Klebsiella pneumoniae Isolates from meningitis: Epidemiology, virulence and antibiotic resistance. Sci. Rep. 2017; 7(1):p6634.
9. Gomez-Simmonds A, Uhlemann AC. Clinical Implications of Genomic Adaptation and Evolution of Carbapenem-Resistant, 2017.
10. Guo Y, Liu F, Zhang Y, Wang X, Gao W, *et al*. Virulence, antimicrobial resistance, and molecular characteristics of carbapenem-resistant Klebsiella pneumoniae in a hospital in Shijiazhuang City from China. International Microbiology, 2023, 1-13.
11. Gupta P, Sarkar S, Das B, Bhattacharjee S, Tribedi P. Biofilm, pathogenesis and prevention-a journey to break the wall: A review. Archives of microbiology. 2016; 198(1):1-15.
12. Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, *et al*. Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. The Journal of Experimental Medicine. 2005; 202(6):761-769.
13. Hasan HM, Jasim HM, Salih GM. Detection of Carbapenem-Resistant Genes and Specific Biofilm Association Genes in K. Pneumoniae Isolated from Medical Samples. The Egyptian Journal of Hospital Medicine. 2022; 89(2):6356-6360.
14. Hullur MS, Natarajan A, Sreeramulu PN. Phenotypic Characterization of Virulence Factors and Antibiogram of Klebsiella pneumoniae Isolates from Various Clinical Samples-A Cross Sectional Study. J Pure Appl Microbiol. 2022; 16(3):1783-1791.
15. Johnson JR, Stell AL. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000; 181:261-272.
16. Lizcano A, Chin T, Sauer K, Tuomanen EI, Orihuela CJ. Early biofilm formation on microtiter plates is not correlated with the invasive disease potential of Streptococcus pneumoniae. Microbial pathogenesis. 2010; 48(3-4):124-130.

17. Nursyam H, Prihanto AA, Warasari NI, Saadah M, Masrifa RE, Nabila NA, *et al*. The isolation and identification of endophytic bacteria from mangrove (Sonneratia alba) that produces gelatinase. IOP Conference Series: Earth and Environmental Science. 2018; 137(1).
18. Bhatia P, Sharma A, George AJ, Anvitha D, Kumar P, Chandra NS. Antibacterial activity of medicinal plants against ESKAPE: An update. Heliyon. 2021; 7(2):e06310
19. Panjaitan NSD, Horng Y, Chien C, Yang H, You R, Soo P. The PTS Components in Klebsiella pneumoniae Affect Bacterial Capsular Polysaccharide Production and Macrophage Phagocytosis Resistance. Microorganisms. 2021; 9(2).
20. Ramakrishnan K, Johnsi PJ, Seetha KS. Clinical isolates of Klebsiella pneumoniae and its virulence factors from a tertiary care hospital. Indian Journal of Microbiology Research. 2019; 6(2):109-112.
21. Sekowska A, Gospodarek E, Janickca G, Jachna-Sawicka K, Sawicki M. Hydrolytic and hemolytic activity of Klebsiella pneumonia and Klebsiella oxytoca. Med Dosw Mikrobiol. 2006; 58(2):135-141.
22. 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. Polish Journal of Microbiology. 2017; 66(4):501-508.
23. Sharma SK, Mudgal NK, Sharma P, Shrngi BN. Comparison of phenotypic characteristics and virulence traits of Klebsiella pneumoniae obtained from pneumonic and healthy camels (Camelus dromedarius). Adv. Anim. Vet. Sci. 2015; 3(2):116-122.
24. Vargas JM, Mochi MM, Nuñez JM, Cáceres M, Mochi S, Del Campo Moreno R, *et al*. Virulence factors and clinical patterns of multiple-clone hypermucoviscous KPC-2 producing K. pneumoniae. Heliyon. 2019; 5(6).
25. Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC, *et al*. Association between *rmpA* and *magA* genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. Clin Infect Dis. 2006; 42:1351-1358.