Original Article

Relationship between Biofilm and the Presence of Drug Resistance Genes in Clinical Isolates of Klebsiella Pneumoniae in Qom Hospitals

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Abstract

Background & Objectives: Klebsiella pneumoniae is a significant cause of nosocomial infections. This bacterium survives in difficult conditions by forming biofilms in hospital equipment and causes severe infections. On the other hand, the emergence and spread of carbapenem resistance among bacteria and biofilm production is a current health concern. There are controversial findings about the relevance of this issue. This study aimed to evaluate the relationship between biofilm formation and carbapenem resistance among clinical isolates.

Materials & Methods: A total of 160 isolates of Klebsiella pneumoniae were collected. Molecular methods were used to detect resistance genes. Subsequently, the ability to produce biofilms in isolates with resistance genes was assessed. Finally, the correlation of biofilm formation among resistant isolates was calculated using $\chi 2$ test.

Results: 79 imipenem-resistant isolates were obtained. 46 isolates (66.66%) containing VIM gene, 36 isolates (52.17%) containing OXA-48 gene, five isolates (7.24%) containing NDM gene, Six isolates (8.69%) containing gene IMP and five isolates (7.24%) also had KPC gene. The results showed a significant correlation between the ability to form biofilms and the presence of carbapenem-resistant genes.

Conclusion: Increased carbapenem resistance in Klebsiella pneumoniae isolates and its association with biofilm formation is severe warning for basic measures to combat this phenomenon.

Keywords: Klebsiella pneumoniae, biofilm, drug resistance, carbapenemase gene, metallobetalactamase gene

Introduction

Due to the emergence and increasing resistance to antibiotics, the treatment of infectious diseases has become a significant concern (1, 2). Reducing the speed of discovery of new antimicrobial drugs leads to the emergence and rapid expansion of resistant isolates, and these resistant isolates pose a serious threat to public health (3, 4). In the past, beta-lactam antibiotics were the mainstay of treatment for gram-negative bacilli-borne infectious diseases. (5, 6) Given these, carbapenems are the next choice against beta-lactam resistant bacteria. Unfortunately, carbapenem-resistant strains of *Klebsiella pneumoniae*

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are emerging and spreading worldwide, which has limited treatment options for various infections with this bacterium (7, 8). Klebsiella pneumonia is one of the most common human pathogens that causes a wide range of community and hospital-acquired infections such as pneumonia, bacteremia, urinary tract infections, respiratory tract and respiratory tract infections (9). In addition to drug resistance, this bacterium is another essential factor in the development of biofilm formation. About 40% of biofilm-producing Klebsiella have been isolated from urine, blood, sputum, and wounds (10, 11). Previous studies have shown an association between antibiotic resistance and biofilm formation. In this case, some researchers have reported that with the production of biofilm, resistance has increased and there is a relationship between the two (12, 13). Other studies have reported that biofilm formation is reduced in high-strength isolates (14). Overall, the relationship between antibiotic resistance and biofilm formation is currently not fully understood and is under investigation (15). This issue is exacerbated when these strains grow with the formation of biofilms and cause a significant increase in resistance to antimicrobial agents (16). Therefore, the aim of this study was to evaluate the relationship between biofilm formation and carbapenem resistance among clinical isolates of *Klebsiella pneumoniae* in Qom hospitals.

Materials & Methods

Bacterial Strains:

This study is cross-sectional. A total of 160 clinical bacteria isolated from clinical specimens were isolated from patients admitted to hospitals in Qom using biochemical tests and were identified as bacterial isolates.

Phenotypic Screening of Carbapenemase:

In this method, swabs of bacteria then two Imipenem discs were placed on the medium at a distance, one of which contains EDTA, and finally the diameter of growth inhibition halos was examined. Increasing the diameter of the stunting halo by more than or equal to 5 mm around the imipenem-EDTA disc relative to the imipenem disc alone indicates the production of metallobetalactamase. To track carbapenemase producers, imipenem discs impregnated with boronic acid were used. In this method, an increase of more than 5 mm of growth inhibition zone around this disc indicates the production of carbapenemase. As a positive control, K. pneumonia ATCC 1705BAA was used.

Detection of Carbapenemase-Related Genes

After genome extraction by boiling method, identification of carbapenem resistance genes by plasmid or chromosomal genes was performed by PCR method. For PCR, Ampilicon (Denmark) Master Mix was used, which contained all the common materials used in PCR and loading buffer. Eppendorf thermal cycling machine (Germany) was also used. Table 1 primers were used in this study. The PCR product was examined by electrophoresis using 1% agarose gel.

Table 1. Primers used to identify carbapenem resistant genes

ESBLs	Primer	PCR-product (bp)
bla $_{IMP}$	Imp-F 5′-GGA ATA GAG TGG CTT AAY TCT C-3′ Imp-R 5′-CCA AAC YAC TAS GTT ATC T-3′	188

ESBLs	Primer	PCR-product (bp)
bla_{VIM}	Vim-F 5′-GAT GGT GTT TGG TCG CAT A-3′ Vim-R 5′-CGA ATG CGC AGC ACC AG-3′	390
bla_{NDM}	NDM-F, 5′-GGTTTGGCGATCTGGTTTTC-3′ NDM-R 5′-CGGAATGGCTCATCACGATC-3-3′	621
bla_{KPC}	KPC-1F5′-CGTTCTTGTCTCTCATGGCC-3′ KPC-1R5′-CCTCGCTGTGCTTGTCATCC-3′	799
bla_{OXA-48}	OXA-48F5′-TTGGTGGCATCGATTATCGG-3′ OXA-48R5′-GAGCACTTCTTTTGTGATGGC-3′	743

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Biofilm assay

To test for biofilm formation, using the microplate phenotypic method, isolates were cultured in TSB medium for 18 hours at 37 °C. Then 200 µl of the medium was transferred to each well from the microplate of 96 sterile houses and incubated for 24 hours at 37 °C. After emptying the wells and rinsing three times with normal saline until the plate was completely dry. Then 200 microliters of 1% violet crystal was poured into the wells for 20 minutes and then washed 3 times with normal saline and finally 200 microliters of ethanol was added to each well and the plate was studied at 595 nm by Eliza Reader. it placed. High adsorption indicates the strength of biofilm formation (17). The ability to produce biofilm was considered in four groups, so that group 1: strong biofilm OD> 0.5 0.5 Group 2: medium biofilm 0.5> OD> 0.3 group 3: Weak biofilm OD <0.3 and group 4: No biofilm formation OD <0.15 was considered.

Results

Carbapenemase producing Isolates

After antibiogram, 79 isolates of *Klebsiella pneumoniae* resistant to imipenem were obtained using phenotypic methods. According to PCR results, out of the total number of imipenem resistant isolates, 69 isolates (87.34%) contained IMP, VIM, NDM, OXA-48 and KPC genes, of which 46 isolates (66.66%) contained VIM gene. 36 isolates (52.17%) contained OXA-48 gene, five isolates (7.24%) contained NDM gene, six isolates (8.69%) contained IMP gene and five isolates (7.24%) contained the KPC gene (Figure1).

Figure 1. Images related to genotypic study of cluster colonies with carbapenem resistant genes

Biofilm producing isolates

All 79 isolates of resistant Klebsiella pneumoniae were examined by phenotypic method to evaluate the biofilm formation strength. 50 isolates (63.29%) had strong biofilm, 20 isolates (25.31%) had moderate biofilm and 9 isolates (39.39%) 11 had poor biofilm (Chart 1).

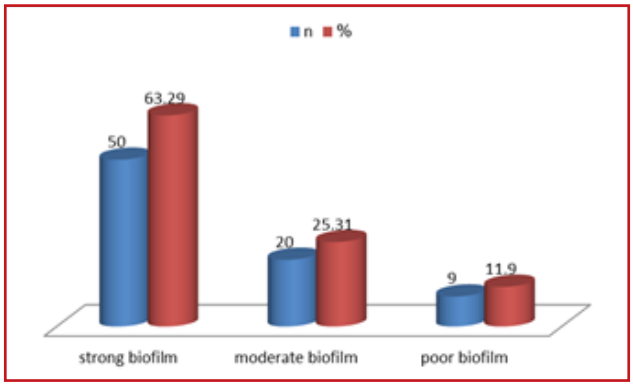

Chart 1. Number and percentage of biofilm formation in resistant isolates

Relationship between gene presence and biofilm formation

No weak biofilm formation was observed in resistant isolates with KPC and NDM genes. According to Table 2, based on Chi-square test, a significant relationship was observed between the ability to form a strong biofilm and the presence of resistance genes (p.value < 0.05).

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Table 2. Number of resistant genes in isolated and biofilm formation capacity

	VIM	IMP	NDM	OXA	KPC
strong biofilm	22	5	5	13	5
Total number of isolates with resistant gene	46	6	5	36	5
p.value	≥ 0.05	≤ 0.05	≤ 0.05	≥ 0.05	≤ 0.05

Discussion

In the present study, the relationship between biofilm formation and carbapenem resistance among clinical isolates of Klebsiella pneumoniae were evaluated. According to our results, the highest gene among blaVIM-resistant isolates was blaOXA and only five isolates had blaKPC and blaNDM genes and blaIMP gene was observed in six isolates.

In the study of Hosseinzadeh et al., it was reported that 10% of the isolates carried the blaNDM-1 gene and the blaKPC gene was not detected in any of the isolates (18). Interestingly, in this study, there was a significant correlation between carbapenem resistance and biofilm. The ability to form biofilms, although it was also seen as a strong biofilm among carbapenem-sensitive isolates, most carbapenem-resistant isolates produced biofilms. In this case, some relationship between biofilm formation and antibiotic resistance has been reported among strains of Klebsiella pneumoniae. For example, among 150 isolates of Klebsiella pneumoniae isolated from sputum and urine, an association between biofilm and the production of broad-spectrum beta-lactamases has been observed (19). In another study, multidrug-resistant *Klebsiella pneumoniae* produced a stronger biofilm than sensitive isolates (20). In the study of Khodadadian et al. In 2018, a significant correlation was seen between the formation of strong biofilm and the prevalence of VIM1 and IMP1 genes (17). In some studies, antibiotic resistance genes are responsible for this correlation and phenomenon, and it has been shown that resistance genes, especially on plasmids, can regulate biofilm formation in Klebsiella (21). Several relationships were found between the ability to form biofilm and antimicrobial resistance, being different for each species. Gentamicin and ceftazidime resistance was related to biofilm formation in Escherichia coli, piperacillin/tazobactam, and colistin in Klebsiella pneumoniae, and ciprofloxacin in Pseudomonas aeruginosa (22).

Conclusion

Acquisition of resistance genes among the bacterial population can be associated with biofilm formation and amplification. Pathogenicity and failure of their treatment. The acquisition of specific antimicrobial resistance can compromise or enhance biofilm formation in several species of Gram-negative bacteria.

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Conflict of Interest

The authors declare that they have no competing interests.

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