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Correlation between *bla*$_{SHV}$ Gene and Biofilm Formation among Beta Lactamase Producing Uropathogenic Isolates from Patients in Erbil City in Iraq

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Abstract

Background and objective: Bacterial resistance has been considered as one of the most serious threats in infectious disease, especially in development countries. The ability of biofilm formation is often associated with antibiotic resistance. The aim of the study is to detect the correlation between the ability of biofilm formation and *SHV* gene, in addition to investigate the prevalence of *SHV* gene among the beta-lactamase producing strains.

Methods: This cross-sectional study was carried out during the period September to December 2017 at Rizgary Teaching Hospital in Erbil. A total of 31 beta-lactamase producing uropathogens were collected**,** identification and antibiotic sensitivity test was done using VITEK-2 automated system. Biofilm formation was determined by 96 well flat bottom microtiter plates. Molecular detection was performed for one of the ESBL genes, the *SHV* gene.

Results: The prevalence of *SHV* gene among the beta-lactamase producing isolates was 29%**.** The findings showed a significant association between the presence of *SHV* gene and the bacterial species (*p*=0.001)**.** In addition 44.4% of *SHV* positive isolates were *K. pneumoniae* pathogens with the ability of moderate or strong biofilm formation which showed to have a significant association (*p*=0.044). From the beta-lactamase producing strains 90.3% appeared to be multi-drug resistant isolates**.**

Conclusion: The *SHV* gene was significantly more present in *K. pneumoniae* in compare to *E. coli* strains. However, most of *SHV* positive isolates were *K. pneumoniae* pathogens with ability of moderate or strong biofilm formation.

Keywords: Biofilm; *SHV* gene; Uropathogens; ESBL

Introduction

Bacterial resistance has been considered as one of the most serious threats in infectious disease, especially in development countries. However a geographical difference has been observed in the antibiotic sensitivity profile of uropathogens. A study from India reported that most of the uropathogenic *E. coli* strains were multi-drug resistant [1].

The World Health Organization (WHO) has classified resistant bacteria according to critically of need for new antibiotics into three groups; critical, high and medium priority. Enterobacteriaceae extended spectrum β-lactamase (ESBLs) enzymes producing bacteria are classified within the critical priority. ESBLs have the ability to hydrolyze broad spectrum of β lactams (third-generation cephalosporins) and monobactams, but do not affect carbapenems or cephamycin [2-4]. Based on their primary structure ESBLs are classified into four classes A, B, C and D enzymes. The class A ESBLs include cefotaximase (*CTX-M*), temoneira (*TEM*) and sulfhydryl variable (SHV) [5]. Additionally it is reported that the most common site for ESBL production are UTIs [6]. Biofilm related infections are often associated with antibiotic resistance [7].

Biofilms are significant colonizers of medical devices for instance urinary, arterial and venous catheters [8]. The main cause of catheter-associated UTI is due to the biofilm formation on the catheter surface [9]. Studies reported that about 80% of all infectious diseases are biofilm related and are also responsible for more than 60% of nosocomial infections. Empirical or antibiotic treatment based on antibiotic sensitivity test is usually inefficient towards infection caused by biofilm producing isolates [10]. Many of the recurrent UTIs are assumed to be caused by biofilm producing uropathogenic isolates [11].

Therefore the aim of the study is to detect the correlation between the ability of biofilm formation and *SHV* gene, in addition to investigate the prevalence of *SHV* gene among ESBL-isolates. Furthermore we observed the antibiotic sensitivity profile of beta-lactamase producing strains.

Materials and Methods

Study design and specimen collection

This cross-sectional study was carried out during the period September to December 2017. A total of 31 beta-lactamase producing uropathogens of *E. coli* and *K. pneumoniae* were collected from patients attending Urology Department at Rizgary Teaching Hospital in Erbil city/ Iraq. The identification and antibiotic sensitivity test was done using VITEK-2 automated system with the ESBL test panel, quality control strains (*E. coli* ATCC 25922).

Biofilm assay

Biofilm formation was determined by the 96 well tissue culture plate (TCP) assay, as described in previous studies [12]. Biofilms were stained with 0.1% crystal violet solution for 15 minutes and the plates were washed in distilled water and dried at room temperature. The optical density (OD) of the biofilms was measured at the wavelength

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630 nm using an ELISA reader. Briefly the isolates were classified into four different categories according to their biofilm-forming ability depending on their 2 ODc (the average OD of the control negative at threefold its standard deviation) of bacteria. The categories were classified as follows, not able to form a biofilm (OD ≤ ODc), weakly able to form a biofilm (ODc<OD ≤ 2xODc), moderately able to form a biofilm (2xODc < OD ≤ 4xODc), strongly able to form a biofilm (4xODc<OD).

Molecular detection of *bla*$_{SHV}$ gene

All isolates which were tested as ESBL positive by VITEK-2 system were screened for detection of *bla*$_{SHV}$. Each bacterial isolate was sub cultured on MacConkey agar for 24 hrs at 37°C. From the plates several single colonies were picked and suspended in 500 mL sterile distal water, and then the bacteria were lysed by heating at 95 °C for 10 minutes. The cell debris were removed by centrifugation at 16000 rpm for 2 minutes. PCR amplification were performed in a final volume of 25 µl containing 12.5 µl Master mix, 2.5 µl of each primer forward and backwards, 5 µl DNA template and 2.5 µl of nuclease free water. Primers have been used in previous studies, *bla*$_{SHV}$ forward primer 5'TTAACTCCCTGTTAGCCA 3' and reverse 5'GATTTGCTGATTTCGCCC 3' have been used with a product size 768 base pairs [13].

The cycling parameters were as followed, initial denaturation at 94 °C for 3 min followed by 30 cycles. Each denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, amplification at 72 °C for 2 minutes and final extension for 10 min [14]. Finally the amplified products were applied to electrophoresis with 1.2% agarose gel in 1XTBE buffer.

Ethical approval

Approval number 181017/78 was obtained from the Ethical committee at College of Pharmacy, Hawler Medical University. In addition, during data collection all of the attended patients were asked for verbal consents.

Statistical analysis

Data analysis was performed using the statistical package for social sciences (SPSS) version 23. The Chi square and Fischer's exact test was used to evaluate any association between two variables a *p* value equal to or less than 0.05 was considered statistically significant.

Results

The antibiotic sensitivity profile of ESBL producing isolates

The antibiotic sensitivity profile of the ESBL isolates indicate that about 90.3% (n=28) were multi-drug resistant (MDR) bacteria, with resistance to more than three classes of antibiotics.

Resistance towards cephalosporin antibiotic class and penicillin class was 100%. Furthermore, the resistance for ciprofloxacin, trimethoprim and gentamycin was moderate with each of 58% (n=18), 58% (n=18) and 64.5% (n=20). In addition nitrofurantoin resistance or intermediate profile was observed in 19.3% (n=6) of the isolates. However, all bacterial strains showed to be sensitive to carbapenem class antibiotics.

The prevalence of *bla*$_{SHV}$ gene and the distribution according to bacterial species

From the total 31 phenotypically confirmed ESBL isolates, 29% (n=9) possessed the resistant *SHV* gene. The gene was present in all *K. pneumonia*e isolates 100% (n=5), while only 15.4% (n=4) of *E. coli* strains harbored the gene. A significant difference has been observed between the presence of *SHV* gene and the bacterial species (p=0.001) as described in Table 1.

Correlation between *bla*$_{SHV}$ gene and biofilm production

About 44.4% (n=4) of the *SHV* positive isolates were moderate or strong biofilm producers, while only 4.5% (n=1) from the *SHV* negative isolates showed moderate or strong biofilm producers. A significant association with $p = 0.044$ is present between the presence of *SHV* gene and the strength of biofilm formation as described in Table 2. Figure 1 showing the PCR results for 31 samples of this study, bands of expected size (768 bp) were seen in positive samples.

Discussion

The emergence of MDR bacteria have changed simple infections into complicated ones. The antibiotic resistance profile of beta-lactamase producing bacteria of the present study was examined, and 90.3% appeared to be MDR isolates. Studies described antibiotic resistance and its significant association with MDR [15,16]. Furthermore, the pathogens were 100% susceptible to last resort antibiotic carbapenems, a finding which is in agreement with other investigations [15,17]. However, in contrast, a recent investigation from Egypt reported 20.4% resistance towards class carbapenems antibiotic [18]. From the current study, resistance of ciprofloxacin was 58% and nitrofurantoin 19.3%. These findings are higher than those reported from investigations in Europe and America which ranged for ciprofloxacin between 4%-39.8% with a susceptibility rate for nitrofurantoin above 99.4% [17,19-22]. Furthermore, studies from India and the Middle East countries described a moderate resistance for ciprofloxacin ranging from 45%-55% and nitrofurantoin 7.5% [14,16,23].

Bacterial species	*SHV* gene			
	Negative No. (%)	Positive No. (%)	Total No. (%)	*p*-value
E. coli	22 (84.6)	4 (15.4)	26 (100)	0.001
K. pneumoniae	0 (0)	5 (100)	5 (100)	
Total	**22 (71)**	**9 (29)**	**31 (100)**	

Table 1: The association between *SHV* gene and the bacterial species.

Biofilm	*SHV* gene			
	Negative No. (%)	Positive No. (%)	Total No. (%)	*p*-value
Non-adherent	8 (36.4)	1 (11.1)	9 (29)	0.044
Weak	13 (59.1)	4 (44.4)	17 (54.8)	
Moderate	1 (4.5)	3 (33.3)	4 (12.9)	
Strong	0 (0)	1 (11.1)	1 (3.2)	
Total	**22 (100)**	**9 (100)**	**31 (100)**	

Table 2: The association between the strength of biofilm formation and *SHV* gene.

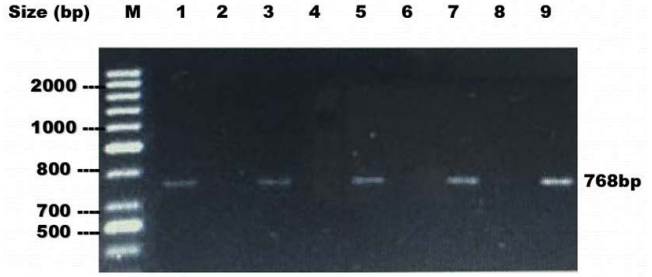

Figure 1: Amplification of *bla*$_{SHV}$ gene from 8 samples of both *E. coli* and *K. pneumoniae*. Agarose gel electrophoresis of PCR amplicons for the target gene. M: 100-1000 bp DNA molecular weight marker, lanes 1-4 are *K. pneumoniae* isolates and lanes 5-8 are *E. coli*.

The prevalence of *SHV* gene among the ESBL producing *E. coli* and *K. pneumoniae* isolates in the study was 29%, which is higher in compare to European countries like Norway with 5% [24,25], the United Kingdom 2.4% (25), Portugal 23.3% [26]. On the other hand, the rate is higher in Kenya 33% [27], India 57.5% [28], Thailand 87.4% [29]. Furthermore, studies from neighboring countries like Iran which included only *K. pneumoniae* samples reported a prevalence of 69.44% [30]. In Iraq, investigations reported a relatively high percentage of *SHV* gene among *K. pneumonia*e which ranged between 55%-87.7% [14,31].

The resistance gene was observed in 100% of *K. pneumonia*e isolates in compare to *E. coli* in which only 15.4% harbor the gene. The findings showed a significant association between the presence of *SHV* gene and the bacterial species (p=0.001) which is in agreement with other investigations [14,32,33]. The *SHV* gene was screened mainly among *K. pneumoniae,* a possible explanation might be that the SHV-1 was categorized as a plasmid-mediated β–lactamase. It is believed that *K. pneumoniae* isolates encode the beta-lactamase enzyme on their chromosomes [14,34]. Furthermore, the present investigation stated that 44.4% of *SHV* positive isolates were *K. pneumoniae* pathogens with the ability of moderate or strong biofilm formation which showed to have a significant association (p=0.044). A possible explanation might be that for *K. pneumoniae*, the presence of *SHV* gene and strength of biofilm production are significantly correlated to each other. A limitation of the study is that from the three genes *SHV, CTX*, and *TEM* of ESBL, PCR was performed only for *SHV* gene.

Conclusion

The *SHV* gene was significantly more present in *K. pneumoniae* isolates in compare to *E. coli.* However, the present investigation stated that most of *SHV* positive isolates were *K. pneumoniae* pathogens with ability of moderate or strong biofilm formation.

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