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Bacterial Biofilm in Ventilator-Associated Pneumonia: A Clinical Concern

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ABSTRACT

Introduction: Development of Ventilator-associated pneumoniae (VAP) presents an intricate chemistry comprising of intubation and invading bacteria which often are tied up with biofilm production.
Aim: To assess the phenotypic and genetic basis of biofilm formation by gram negative bacilli isolated from mechanically ventilated and VAP developed patients.
Materials and Methods: Endotracheal aspirate obtained from 40 mechanically ventilated patients was inoculated by a semi quantitative method. Isolation and identification of gram negative bacilli was done according to standard bacterial protocol followed by antibiotic susceptibility test and biofilm assessment by micro titer method. Presence of biofilm genes was studied by molecular method.
Result: Amongst 40 enrolled hospitalized patients, 12.5% of them developed VAP. A. baumannii (n=68) was the commonest organism followed by P. aeruginosa (n=18), K. pneumoniae (n=32) and E. coli (n=12). Presence of multi drug (MDR) and extensive drug resistance (XDR) was evident in A. baumannii and P. aeruginosa comparative to K. pneumoniae and E. coli isolates. Moderate or strong biofilm production was an overt feature. Presence of bap gene was marked in 57.4% A. baumannii whereas, 11.1% P. aeruginosa displayed algC gene. Manifestation of genes fimH, ang43 and csgA was evident in 33.3%, 66.7% and 25% E. coli isolates respectively. Gene type 3 was present in all K. pneumoniae isolates while, mrkA and fimK genes were shown in 65.6% and 81.2% isolates respectively by multiplex PCR.
Conclusion: Drug resistance phenotypes require specific national and regional therapeutic studies focusing on antimicrobial stewardship programs. Presence of biofilm requires routine surveillance of VAP, to track endemic VAPs.

Key words: Ventilator-associated pneumonia, Mechanical ventilation, Bacteria, Antibiotic-resistant, Biofilm

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INTRODUCTION

Clinico-microbiological parameters play a vital role to define Ventilator-associated pneumonia (VAP). The condition is defined clinically as pneumonia that occurs 48–72 hours or thereafter following endotracheal intubation, characterized by the presence of a new or progressive infiltrate, signs of systemic infection (fever, altered white blood cell count), changes in sputum characteristics, and microbiologically by detection of a causative agent [1-3]. VAP is the second most common nosocomial infection in the mechanically ventilated patients admitted to the intensive care unit (ICU) [4,5]. The complex interplay between the endotracheal tube, presence of risk factors, virulence of the invading bacteria and host immunity largely determine the development of VAP.

Infection in VAP develops by direct entry of bacteria to lower respiratory tract, which may be innate flora of oropharynx or those present in the hospital *via*: (a) micro aspiration, which can occur during intubation itself; (b) development of a biofilm overloaded with bacteria (typically Gram-negative bacteria and fungal species) within the endotracheal tube; (c) pooling and trickling of secretions around the cuff; and (d) impairment of mucociliary clearance [1].

The type of organism that causes VAP usually depends on the duration of mechanical ventilation. In general, early VAP is caused by pathogens that are sensitive to antibiotics, whereas late onset VAP is caused by multi-drug resistant and more difficult to treat bacteria. Geographical variation is being detected for the

exact prevalence of MDR organisms and even within institutions at one place [1,3].

Bacterial biofilms found on the surface of any medical devices plays an important role in retaining infectious material. In fact, the surfaces of all medical and dental devices are normal targets for colonization by complex microbial communities [6,7]. The earlier concepts of biofilm that it develops through series of steps have been extended by the exact nature of its formation in the light of bacterial structures involved. Adhesiveness and biofilm-forming ability of gram positive or negative bacteria play a pivotal role in medical device-associated infections and literature reveals that genetic determinants involved in the adherence should be taken into account for biofilm formation [8,9].

We aimed to study the phenotypic and genetic basis of biofilm formation by *Escherichia coli, Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from mechanically ventilated and VAP developed patients.

MATERIALS AND METHODS

This prospective study was conducted from May 2015 to March 2016, in a respiratory ICU of a University Teaching hospital. Forty mechanically ventilated adult patients (≥ 20 years old) were enrolled in this study and observed for the development of first episode of VAP. From all these patients endotracheal specimen was obtained four times i.e., after 48 hours, 72 hours, 7th day and 10th day of intubation and each time the specimen was inoculated by semi-quantitative method on a selective and non-selective bacteriological media. When bacteria was grown with bacterial count of 10^5 CFU/ml, the culture was considered positive and all isolates were identified using conventional microbiology methods [10,11].

Antibiotic susceptibility

Antibiotic susceptibility of all isolates of was performed according to the CLSI [12] by disc diffusion test (Kirby-Bauer) on Muellar Hinton agar. The result of antibiotic susceptibility test was interpreted on the basis of CLSI standard. The following antimicrobial agents were used to evaluate susceptibility of isolates: Gentamicin (30 µg), Ofloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), Trimethoprim-Sulfamethoxazole (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Colistin (30 µg), Piperacillin-Tazobactam (10 µg), Ciprofloxacin (5 µg), Cefotaxime (30 µg) and Amikacin (30 µg).

Biofilm formation

Biofilm was performed first by micro titer-plate by first inoculating bacterial isolates in 10 ml of Tryptic soy broth (TSB) medium containing 1% glucose followed by incubating the medium for 24 hours at 37-35°C. After incubation, bacterial growth was diluted and suspension was matched equivalent to 0.5 McFarland. From this diluted suspension 200 µl was added into 96-well sterile plate (the negative control well comprised only TBS with 1% glucose), and incubated further for 24-48 hours at 37°C without shaking. The contents of each well was emptied after incubation and each well was washed gently with PBS (2%) four times, followed by use the 2% sodium acetate to fix the biofilms formed in the wells, which were then stained with crystal violet. Excess color was washed with deionized water and plates were kept at the room temperature to dry. The optical density of the biofilm sticking to the well was read by the ELISA and analyzed as depicted below in Table 1 [13].

Table 1: Analysis of the biofilm adherence

Mean OD value	Adherence	Biofilm formation
$OD_c \geq OD$	none	none
$2OD_c \geq OD \geq OD_c$*	weak	weak
$4OD_c \geq OD \geq 2OD_c$	moderate	moderate
$OD > 4OD_c$	strong	strong

*OD_c: Optical density of control well

Biofilm was detected on the basis of the presence of genes implicated in biofilm formation in *E. coli* (*fimH, csgA* and *ang43*) [14,15], *K. pneumoniae* (*fimK*, *type3*, *mrkA*) [14,16] *A. baumannii* (*bap*) [17] or *P. aeruginosa* (*algC*) [18] by Polymerase chain reaction (PCR) as described previously [14-17]. Table 2 describes the primers for each gene and the PCR condition respectively.

RESULTS

A total of 40 hospitalized patients in the ICU who had used mechanical ventilation for more than 48 hours were enrolled in the study and comprised: 24 (60%) males and 16 (40%) females. The incidence of VAP in the current study was 12.5% (n=5), which was determined based on the clinical and microbiological criteria for the VAP diagnosis and was mostly observed as late onset in all five patients. Table 3 displays microbiological etiology observed in VAP as well as non VAP patients. Amongst gram negative pathogens isolated and identified by phenotypic biochemical methods, the most common organism isolated was *Acinetobacter baumannii* (n=68) followed by *Pseudomonas aeruginosa* (n=18), *Klebsiella pneumoniae* (n=32), and *Escherichia coli* (n=12). These bacterial pathogens were isolated in single or in combination from each patient. Frequency of aforementioned pathogens in VAP patients or non VAP mechanically ventilated patients did not differ significantly.

Very high frequency (52% to 100%) of resistance was seen by *A. baumannii* towards most of the antibiotics tested. Though *P. aeruginosa* had comparatively more resistance level towards most of the antibiotics tested, however had low resistance towards piperacillin-tazobactum, ceftazidime and amikacin. *K. pneumoniae* showed antibiotic resistance for imipenem, and all cephalosporins, the three drugs most commonly used in ICU settings. Table 4-5 shows the result of antimicrobial susceptibility test. Amongst *A. baumannii*, 13 (68.4%) isolates showed multi resistance (MDR), while 5 (26.3%)

Table 2: Description of primers utilized in the present investigation

Gene	Primer	Annealing temperature (°C)	Product size (bp)
bap-F	5′-TAGGGAGGGTACCAATGCAG-3′	61	400
bap-R	5′-TCATGATTTGATGCTGCAGCG-3′		
FimH-F	5′-CGCCTGGTCCTTTGCCTGCA-3′	69	817
FimH-R	5′-CTGCACGTTGCCGGCGGTAA-3′		
type3-F	5′-GGGACAGATACGCGTTTGA-3′	52	817
type3-R	5′-GGCCTAACTGAACGGTTTGA-3′		
mrkA-F	5′-GTTAACGGCCAGGGCAGCGA-3′	69	382
mrkA-R	5′-AGGTGAAACGCGCGCCATCA-3′		
FimK-F	5′-GCGGCTGGCTGGGGTGAAAAAG-3′	54	597
FimK-R	5′-AGTCGACGCGCCGGAAAGATAACG-3′		
algC-F	5′-TTAGAACCGGGTGAGCGTTTAGCA-3′	54	1781
algC-R	5′-AGATGCTGATCTTGTGGCATTGCG-3′		
ang43-F	5′-TTCCGGGAAGACGGTGAA-3′	58	144
ang43-R	5′-TTCTGGGTGAGTGTGGTGTT-3′		
csgA-F	5′-TGGCAGGTGTTCCTCAG-3′	58	146
csgA-R	5′-GTCAGAGTTACGGGCATCAGTT-3′		

Table 3: Frequency of Gram negative bacilli isolated from VAP and non-VAP patients

Pathogens	Total	Frequency of pathogens in two groups	
		VAP (n; %)	Non VAP (n; %)
Acinetobacter baumannii	68	19 (27.94)	49 (72)
Pseudomonas aeruginosa	18	6 (33.33)	12 (66.66)
Klebsiella pneumoniae	32	11 (34.37)	21 (65.62)
Escherichia coli	12	4 (33.33)	8 (66.66)

Table 4: Result of antibiotic susceptibility of pathogens isolated in this study

Antibiotics	*Acinetobacter baumannii (%)*	*Klebsiella pneumoniae (%)*	*Pseudomonas aeruginosa (%)*	*E. coli (%)*
Gentamicin	57.9	54.5	83.3	50
Ofloxacin	94.7	100	100	100
Imipenem	100	100	100	50
Meropenem	631	54.5	83.3	-
Co-trimoxazole	2.84	63.6	100	25
Ceftriaxone	100	100	100	100
Amikacin	52.6	36.4	33.3	0
Ceftazidime	100	100	16.7	100
Colistin	0	-	0	-
Piperacillin tazobactum	2.84	-	716	-
Ciprofloxacin	100	100	100	100
Cefotaxime	100	100	-	100

Table 5: Manifestation of biofilm by bacterial pathogens

Pathogen	Biofilm genes	Frequency [as percentage (%)] of isolates positive for respective genes on PCR	Frequency [as percentage (%)] of isolates positive [strong or moderate] for biofilm phenotypically
A. baumannii	*bap*	57.4	60.2
K. pneumoniae	*type3*	100	67.7
	mrkA	65.6	
	fimK	81.2	
E. coli	*fimH*	33.3	8.3
	ang43	66.7	
	csgA	25	
P. aeruginosa	*algC*	11.1	44.4

were Extensive drug resistance (XDR). Among *Klebsiella pneumoniae* 4 (36.3%) isolates were found MDR and other 4 isolates as XDR. Only one (16.6%) isolate of *P. aeruginosa* was observed as XDR, while 5 (83.3%) isolates were

observed as MDR. None of *E. coli* isolates were seen as MDR, while only one (25%) was found as XDR.

Table 5 depicts the presence of biofilm genes in the bacterial pathogens. Overall, more than 60% of A. baumannii and K. pneumoniae isolates showed presence of biofilm genes.

DISCUSSION

Ventilator associated pneumoniae is a nosocomial infectious affliction which is presented after 48 hours to 72 hours of stay in the hospital [19]. In the present investigation endotracheal aspirate from 40 ICU admitted and mechanically ventilated patients was cultured for any gram negative organism and their capacity to form biofilm was assessed phenotypically as well as genotypically. Endotracheal aspirate collected on various days of stay in the hospital, revealed presence of *A. baumannii* (n=68), *K. pneumoniae* (n=22), *Pseudomonas aeruginosa* (n=18) and *E. coli* (n=12) with colony count of more than 10^5 CFU/ml. *A. baumannii* was not only the commonest microorganism in mechanically ventilated patients but also patients of VAP. Our report is analogous to other published studies from United States and other countries [2,20-22] whereby most frequent bacterial agent associated with VAP has been gram negative organisms. However, frequency of bacterial isolates differ geographically and even local regions. All the above research studies performed revealed either *K. pneumoniae* or *P. aeruginosa* or *A. baumannii* as the most frequent bacterial isolates.

The present study evaluated the antibiotic susceptibility of all bacterial isolates by Kirby Bauer method using the standard guidelines of CLSI. Most of the gram negative organisms were resistant to cephalosporins, quinolones and carbapenems. Our study is compatible to a research performed in Pakistan which found 95.6% isolates resistant towards imipenem. Resistance pattern of *A. baumannii* towards several antibiotics was found similar to another study performed by Ibrahim and colleagues in year 2016 [23]. The only antibiotic to which this organism did not develop resistance was colistin. *K. pneumoniae* was found as the second most common bacterial agent of VAP in our study. This organism was found mostly resistant to imipenem, ceftriaxone, ceftazidime, cefotaxime and ciprofloxacin. Almost similar finding has been reported from Bangladesh [24] and other research study [25] which found this organism to be resistant to not only the above mentioned antibiotics but also to gentamicin and amikacin. *P. aeruginosa* showed high level resistant to imipenem (83.3%), ofloxacin (83.3%) and low level resistance towards piperacillin-tazobactum (5.6%) in our investigation. Other published studies conducted in year 2010 [26] and 2014 [27] furnishes high level resistance of this organism to most of the antibiotics, specially 45% of their isolates were resistant to piperacillin-tazobactum [27] in comparison to our results. *E. coli* is another gram negative organism which has developed resistance to many antibiotics and this is a matter of concern as resistance genes are easily transferable to other strains. In our study, *E. coli* isolates demonstrated moderate resistant to extended spectrum cephalosporin (28% to 38.3%) and aminoglycosides (8.3%-38%). Our results are compatible to another published study [28] but the results of others study showed widespread resistance (51.1%-91.2%) of the isolates to all the antibiotics, except nitrofurantoin with resistance rate of 7.3% [29].

Biofilms are common concern in medicine as they develop commonly on medical devices and they can also form on living tissues, as in the case of endocarditis. Biofilms grow slowly, in one or more locations, and biofilm infections are often slow to produce overt symptoms [30]. In our study 11.1% *P. aeruginosa* isolates were positive for *algC* gene. Though presence of this gene has not been studied till now in VAP or other mechanically ventilated patients, but other studies on urinary tract catheter, cystic fibrosis and burn patients show involvement of this gene in biofilm production [31,32]. We studied the presence of biofilm genes in *K. pneumoniae* and *E. coli* also. All *K. pneumoniae* isolates were positive for *type3* gene while, *fimK* was observed in 96.8% isolates and *mrk* being seen in 65.6% of isolates. Presence of these above mentioned genes and antibiotic resistance among *K. pneumoniae* isolates shows a concern for mechanically ventilated patients and particularly for VAP developed patients. Other studies on the presence of these genes in this organism also supports our results [15,33]. These published research showed presence of these genes in high number of isolates obtained from endotracheal aspirate or even cerebrospinal fluid, blood, urinary catheter. Presence of genes *fimH,* and *csgA*, was observed in high number of isolates with 66.7%, 75% while *ang43* was seen in only 33.3% respectively in our study. No study is available on the presence of these genes in *E. coli* obtained from VAP or mechanically ventilated patients. Ours is the first kind of investigation on such aspect. Gene *bap* has been widely studied in *A. baumannii*. *A. baumannii* Bap is a key factor in biofilm formation and thus may have a role in persistence in the hospital environment and also in infection [34,35]. Approximately 57% of *A. baumannii* isolates in our study were positive for this gene. Our earlier study on this organism though isolated from various clinical specimens but not endotracheal aspirate showed 43% of the isolates were strong biofilm-producer and 32% of the isolates as moderate biofilm-formers with frequency of *bap* as 92% [35]. Other studies performed in Iran showed 30% frequency of this gene in their *A. baumannii* isolates [36,37].

Biofilms are highly organized microbial communities which *in vivo* play an important part in evading the defense mechanism and obstinate the antimicrobial therapy. The precise link between biofilm productions in mechanically ventilated patients is still obscure apart from some limited studies which have described role of a specific pathogen or virulence factor. The present study, thus, showed that majority of the pathogens isolated from endotracheal aspiration of mechanically ventilated

patients including VAP, were resistant to most of the empirical therapeutic agents and not only possessed biofilm genes but also capability to produce biofilm. These observations suggest that biofilm formation could contribute lower respiratory infection and help the organisms evade antimicrobial pressure and emergence of multidrug or extensive drug resistant microorganism. The condition may lead to development of nosocomial infection too.

CONCLUSION

In summary, we provided data regarding the presence of biofilm genes in the high to moderate antibiotic resistant gram negative bacteria causing VAP which reveals predilection towards biofilm and is a concern in medical practice. It also necessitates the development of new strategies to impair their ability to persist in biofilm environment.

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REFERENCES

1. Kalanuria AA, Zai W, Mirski M, et al. Ventilator-associated pneumonia in the ICU. Crit Care 2014; 18:208.
2. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis 2010; 51:S81-S87.
3. Safdar N, Crnich CJ, Maki DG. The pathogenesis of ventilator-associated pneumonia: Its relevance to developing effective strategies for prevention. Resp Care 2005; 50:725-741.
4. Hunter JD. Ventilator associated pneumonia. BMJ 2012; 344:3225.
5. Afshari A, Pagani L, Harbarth S. Year in review 2011: Critical care-infection. Crit Care 2012; 16:242.
6. Hassan A, Usman J, Kaleem F, et al. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 2011; 15:305-311.
7. Longo F, Vuotto C, Donelli G. Biofilm formation in *Acinetobacter baumannii*. New Microbiol 2014; 37:119-127.
8. Lee HW, Koh YM, Kim J, et al. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect 2008; 14:49-54.
9. Tolker-Nielsen T, Molin S. Spatial organization of microbial biofilm communities. Microb Ecol 2000; 40:75-84.
10. Forbes BA, Sahm DF, Weissfeld AS. Bailey Scott's diagnostic microbiology 2014; 307-315.
11. Collee JG, Mackie TJ, McCartney JE. Mackie & McCartney practical medical microbiology. Edinburgh: Charchil Livingstone 1989.
12. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing 2011; 17.
13. Christensen GD, Simpson W, Younger JJ, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 1985; 22:996-1006.
14. Murphy CN, Mortensen MS, Krogfelt KA, et al. Role of *Klebsiella pneumoniae* type 1 and type 3 fimbriae in colonizing silicone tubes implanted into the bladders of mice as a model of catheter-associated urinary tract infections. Infect Immun 2013; 81:3009-3017.
15. Cruz-Córdova A, Esteban-Kenel V, Espinosa-Mazariego K, et al. Pathogenic determinants of clinical *Klebsiella pneumoniae* strains associated with their persistence in the hospital environment. Boletín médico del Hospital Infantil de México 2014; 71:15-24.
16. Alcántar-Curiel MD, Blackburn D, Saldaña Z, et al. Multi-functional analysis of *Klebsiella pneumoniae* fimbrial types in adherence and biofilm formation. Virulence 2013; 4:129-138.
17. Deighton MA, Capstick J, Domalewski E, et al. Methods for studying biofilms produced by *staphylococcus epidermidis*. Methods Enzymol 2001; 336:177-195.
18. Struve C, Bojer M, Krogfelt KA, et al. Characterization of *Klebsiella pneumoniae* type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. Infect Immun 2008; 76:4055-4065.
19. Hosseini Shirazi M, Alipour A, Firouzian A, et al. Comparing sedation receive as blouse and infusion at incidence of ventilator-associated pneumonia in intubated traumatic patients in ICU. SSU J 2017; 24:818-827.
20. Hortal J, Muñoz P, Cuerpo G, et al. Ventilator-associated pneumonia in patients undergoing major heart surgery: An incidence study in Europe. Crit Care 2009; 13:R80.
21. Charles MP, Kali A, Easow JM, et al. Ventilator-associated pneumonia. Australasian Med J 2014; 7:334-344.
22. Torres A, Ferrer M, Badia JR. Treatment guidelines and outcomes of hospital-acquired and ventilator-associated pneumonia. Clin Infect Dis 2010; 51:S48-S53.
23. Akers KS, Chaney C, Barsoumian A, et al.

Aminoglycoside resistance and susceptibility testing errors in *Acinetobacter baumannii-calcoaceticus* complex. J Clin Microbiol 2010; 48:1132-1138.
24. Ahmed W. Microorganisms related with ventilator associated pneumonia (VAP) and their antibiotic sensitivity pattern. J Rawalpindi Med Coll 2014; 18:45-48.
25. Tsakiridou E, Makris D, Daniil Z, et al. *Acinetobacter baumannii* infection in prior ICU bed occupants is an independent risk factor for subsequent cases of ventilator-associated pneumonia. Biomed Res Int 2014; 2014:193516.
26. Ahsan AA, Barai L, Faruq MO, et al. Antibiotic resistance pattern among bacteria causing ventilator associated pneumonia in an intensive care unit of Bangladesh. Bangladesh Crit Care J 2016; 4:69-73.
27. Golia S, Sangeetha K, Vasudha C. Microbial profile of early and late onset ventilator associated pneumonia in the intensive care unit of a tertiary care hospital in Bangalore, India. J Clin Diag Res 2013; 7:2462.
28. Khezri HD, Gorji MAH, Morad A, et al. Comparison of the antibacterial effects of matrica & Persica and chlorhexidine gluconate mouthwashes in mechanically ventilated ICU patients: A double blind randomized clinical trial. Rev Chilena Infectol 2013; 30:368-373.
29. Olorunmola FO, Kolawole DO, Lamikanra A. Antibiotic resistance and virulence properties in *Escherichia coli* strains from cases of urinary tract infections. Afr J Infect Dis 2013; 7:1-7.
30. Costerton JW, Stewart PS, Greenberg EP, et al. Bacterial biofilms: A common cause of persistent infections. Sci 1999; 284:1318-1322.
31. Mulcahy LR, Isabella VM, Lewis K. *Pseudomonas aeruginosa* biofilms in disease. Microb Ecol 2014; 68:1-12.
32. Laverty G, Gorman SP, Gilmore BF. Biomolecular mechanisms of *Pseudomonas aeruginosa* and Escherichia coli biofilm formation. Pathog 2014; 3:596-632.
33. Sahu PK, Iyer PS, Barage SH, et al. Characterization of the algC gene expression pattern in the multidrug resistant *Acinetobacter baumannii* and correlation with biofilm development on abiotic surface. Sci World J 2014.
34. Brossard KA, Campagnari AA. The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. Infect Immun 2012; 80:228-233.
35. Fallah A, Rezaee MA, Hasani A, et al. Frequency of *bap* and cpaA virulence genes in drug resistant clinical isolates of *Acinetobacter baumannii* and their role in biofilm formation. Iran J Basic Med Sci 2017; 20:849.
36. Neamati F, Firoozeh F, Saffary M, et al. The prevalence of uropathogenic *E. coli* and detection of some virulence genes isolated from patients referred to Kashan Shahid-Beheshti hospital during 2012-2013. Feyz J Kashan Univ Med Sci 2014;18:12-15
37. Liu W, Vives-Bauza C, Yamamoto A, et al. PINK1 defect causes mitochondrial dysfunction, proteasomal deficit and α-synuclein aggregation in cell culture models of Parkinson's disease. PloS One 2009; 4:e4597.