IJMS
Vol 41, No 5, September 2016

Original Article

SDS-PAGE Analysis of the Outer Membrane Proteins of Uropathogenic *Escherichia coli* Isolated from Patients in Different Wards of Nemazee Hospital, Shiraz, Iran

Behzad Dehghani[1], MS;
Mohammad Mottamedifar[1,2], PhD;
Hossein Khoshkharam-Roodmajani[1], MS;
Amir Hassanzadeh[1], MS;
Kamyar Zomorrodian[3], PhD;
Amir Rahimi[4], PhD

[1]Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;
[2]Shiraz HIV/AIDS Research Center Shiraz University of Medical Sciences, Shiraz, Iran;
[3]Basic Sciences in Infectious Disease Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;
[4]Department of Molecular Medicine, School of Advanced Medical Science and Technology, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:
Mohammad Motamedifar, PhD;
Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences,
Zip Code: 71348-45794, Shiraz, Iran
Tel/Fax: +98 71 32304356
Email: motamedm@sums.ac.ir.
Received: 06 June 2015
Revised: 22 August 2015
Accepted: 06 September 2015

What's Known

- Outer membrane proteins (OMPs) of *E. coli play* a significant role in antibiotic resistance and pathogenesis.
- OMP banding patterns obtained by SDS-PAGE are categorized in 3 groups (OMP-I, OMP-α, and OMP-β).
- OMP typing can determine the source of infection and the diversity of isolates collected from different hospital wards.

What's New

- There is no information about *E. coli* OMP types, especially in Iran.
- OmpA and OmpC were the most prevalent OMPs in our uropathogenic *E. coli* isolates.
- ABC type band pattern frequency in our hospital isolates was lower than that for the isolates from our OPD patients.

Abstract

Background: Outer membrane proteins (OMPs) constitute the main structure and about half of the cell wall of Gram-negative bacteria. The OMPs of *Escherichia coli* (*E. coli*) play an important role in its drug resistance. Previous studies have shown that the OMPs of *E. coli* enhance its pathogenic effects by helping the bacterium to evade the immune defense and promote its adsorption to host cells. We sought to compare *E. coli* isolates collected from different hospital wards and to perform a primary investigation of the association between the serotypes and profiles of their OMPs. We also aimed to detect the diversity of the *E. coli* isolates from the hospitalized patients.

Methods: A total of 115 isolates of *E. coli* were collected from patients hospitalized in Nemazee Hospital, Shiraz, Iran. After biochemical and serological tests, OMPs were extracted by using glass beads and N-Lauroylsarcosine sodium. OMP typing was done by 10% SDS-PAGE and Coomassie brilliant blue staining. In terms of the number of protein bands, OMP-I was detected with 2 bands, OMP-α with 3 bands, and OMP-β with1 band.

Results: Of the 115 isolates, 103 were OMP-I and 12 were OMP-α; none of the isolates belonged to OMP-β. Our statistical analyses showed a relationship between OMP patterns and other factors, including hospital wards and source of samples. Serotyping showed that the majority of the isolates were O128.

Conclusion: Our results demonstrated some similarities between the OMP band patterns of the analyzed groups of *E. coli*. Of all the OMPs in the isolates from the hospitalized and outpatient department patients, OmpA and OmpC were the most prevalent proteins in the outer membrane of the studied uropathogenic *E. coli*.

Please cite this article as: Dehghani B, Mottamedifar M, Khoshkharam-Roodmajani H, Hassanzadeh A, Zomorrodian K, Rahimi A. SDS-PAGE Analysis of the Outer Membrane Proteins of Uropathogenic *Escherichia coli* Isolated from Patients in Different Wards of Nemazee Hospital, Shiraz, Iran. Iran J Med Sci 2016;41(5):399-405.

Keywords ● Escherichia coli ● Bacterial outer membrane proteins ● Urinary tract infections ● Electrophoresis ● Polyacrylamide gel ● Bacterial typing techniques

Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases, an important public health problem, and a major cause

of morbidity and mortality in humans.[1-3] UTI comprises cystitis (infection of the bladder) and pyelonephritis (infection of the kidney) and is defined as colonization of microorganisms in the urinary tract.[3] Uropathogenic *Escherichia coli* (*E. coli*) is responsible for 70 to 90% of the cases of community-acquired UTI and approximately 40% of all the cases of nosocomial UTI in the United States.[4,5] Various virulence factors are responsible for the pathogenicity of uropathogenic *E. coli* strains in humans. Some of these factors are related to the bacterial envelope.

The cell-surface of *E. coli*, like other members of *Enterobacteriaceae*, consists of 3 layers: the cytoplasmic membrane, a peptidoglycan layer, and the outer membrane. The outer membrane consists of lipids, polysaccharides, and proteins.[6-9] Outer membrane proteins (OMPs) are the main structure and comprise half of the cell wall of a Gram-negative bacterium and act as a physical barrier at the bacterial surface, enabling the bacterium to be resistant against bile salts, antibiotics, proteolytic enzymes, and other hostile factors.[10-13]

Except for plasmid-coded proteins, OMPs are encoded by bacterial chromosomes. The difference between various classes of OMP-encoding genes accounts for the difference between major OMPs.[14]

Previous studies have indicated the diversity and the broad functions of OMPs. These functions include the uptake of nutrients, nucleosides, phosphate, and maltose and transport of maltodextrins. OMPs also act as receptors for the uptake of ferric iron, vitamin B12, fatty acids, bacteriophages, and bacteriocins. Furthermore, OMPs help the pathogen to evade the host immune defense and promote its adsorption to host cells.[15] Drawing upon sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), previous studies have reported that the major subunits of OMPs comprise ~30–40 kDa proteins and characterized their genetic and serotype relationships.[14,16] OMP typing can also determine the source of infection and the diversity of *E. coli* isolates collected from different wards in hospitals.

We sought to compare isolates collected from different hospital wards and to conduct a primary investigation of the association between the serotypes and profiles of their OMPs. We also aimed to detect the diversity of the *E. coli* isolates collected from the hospitalized patients.

Patients and Methods

Isolates

In a cross-sectional study from April to July 2012, a total of 115 *E. coli* isolates were obtained from patients with UTI in Nemazee Hospital, Shiraz, Iran. The bacteria were identified and confirmed using standard methods. After gross and microscopic examinations, the urine was cultured on MacConkey agar and Xylose lysine deoxycholate agar (Merck, Germany). The cultured plates were incubated at 37°C for 24 hours until the occurrence of growth. Up to 5 lactose-fermenting colonies were selected separately and subjected to routine biochemical tests. The samples were then stored and sub-cultured for further analysis.

E. coli Serotyping

All the isolates were serotyped via the O serotype slide agglutination test using a diagnostic *E. coli* antisera kit (Bahar Afshan, Iran) according to the manufacturer's guidelines.

Outer Membrane Protein Extraction

Pure cultures of the *E. coli* samples were grown at 37 °C in 2 mL of the LB broth medium (Merck, Germany) under constant shaking at 200 rpm for 6–7 hours. For sub-culturing, 100 µL of the culture was inoculated into 10 mL of the LB medium and incubated overnight at 37 °C with constant shaking. Then, the cells were harvested by centrifugation at 10000 ×g for 10 minutes and the precipitate was suspended in 300 µL of 10 mmol/L of HEPES (Sigma, Germany). The cells were lysed using glass beads, and centrifuged at 10000 rpm/min for 15 minutes at 4 °C.

A total of 750 µL of a 2% Lauroylsarcosine sodium (Sigma, Germany) solution was added to the supernatant. After 20 minutes of incubation at room temperature, the mixture was centrifuged at 10000 rpm for 1 hour at 4 °C.

The precipitate was dissolved in 3 mL of 10 mmol/L of HEPES and an equal volume of a 2% Lauroylsarcosine sodium solution. The above step was repeated twice. The precipitate was dissolved in an appropriate amount of HEPES (10 mmol/L) and stored at −20 °C.

Analysis of the Outer Membrane Proteins

The OMP samples were analyzed using10%SDS-PAGE. The protein concentrations of the prepared OMPs were measured by the method of Lowry et al.[17] SDS-PAGE was performed with 4.8% stacking and 12% separating gel after the OMP preparation was solubilized at 100 °C for 5 minutes in 0.05 M of Tris-HC1 buffer (2.5% SDS, 5% 2-mercaptoethand, 25% glycerol, and 0.03% bromophenol blue). Protein bands were detected after 1 hour of staining with 0.25% Coomassie brilliant blue R250 (Sigma, Germany) using low molecular weight protein markers (Fermentas, Germany). Protein markers (10–200 kDa) were

used for SDS-PAGE. The gel images were then scanned and analyzed by Image J software.

Protein bands can appear as 30–43 kDa protein bands.[14,16] According to the number of protein bands, the OMP type in the present study was determined as OMP-I with 2 bands, OMP-α with 3 bands, and OMP-β with 1 band.

Statistical Analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS, Inc., Chicago, IL, U.S.A.). Comparisons of the categorical variables were done using the chi-square test. The level of significance was set at a $P<0.05$ using 2-sided comparisons.

All data were collected anonymously in accordance with legal requirements regarding data protection and medical confidentiality. Approval was obtained before the commencement of the study from the Ethics Committee of the Faculty of Human Research.

Results

E. coli Samples

Totally, 115 samples were isolated from patients with UTI from Nemazee Hospital, Shiraz, Iran, in 2012. Among the patients, 41.6% were hospitalized and 58.4% were from the outpatientdepartment (OPD). The majority of the hospitalized patients were in the internal ward and only 4 (3.5%) patients were in the neurology ward (figure 1).

Serological Results

The results of the serological test showed that 63.5% of the samples were nontypeable by the used kit and the other samples were categorized in serogroups (O26, O55, O86, O127, O44, O125, O128, O20, and O114). figure 2 shows the serological results and the percentages in each group.

Banding Results of the Outer Membrane Proteins

The OMPs of the *E. coli* isolates were investigated via SDS-PAGE and grouped in 3 categories (table 1).

The patterns obtained for the major OMPs with apparent molecular weights between about 30 and 42 kDa are shown in figure 3.

Analysis of the SDS-PAGE results showed that 89.5% of the samples were OMP-I. In OMP-I type, 2 sub-types were determined: AB and BC. No significant differences were detected between the frequencies of the 3 *E. coli* banding patterns (table 1). The OMP

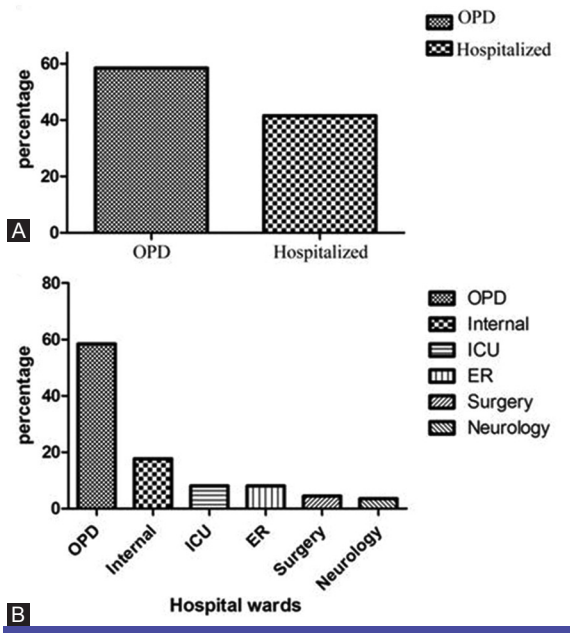

Figure 1: (A) presents the frequency of the studied samples in the hospitalized and outpatient department (OPD) patients. (B) shows the frequency of the studied samples in each ward of the hospital compared to the OPD patients.

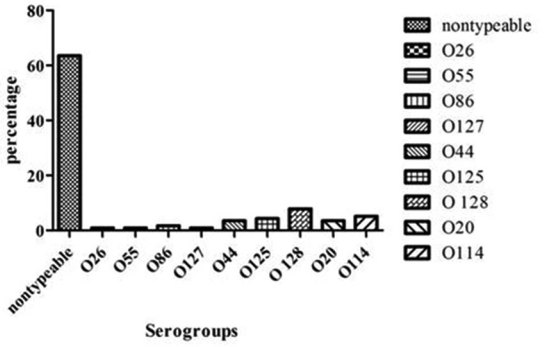

Figure 2: These are the percentages of the serotype groups of the studied uropathogenic *E. coli* isolates.

banding analysis in the hospital wards showed a difference in the major band types. In the internal ward patients, 90% of the isolates were BC type, 5% were ABC type, and 5% were AB type. The major OMP banding was AB (50%) in the emergency department and BC (80%) in the intensive care unit. In all the 3 wards (internal ward, emergency department, and intensive care unit), the major serological results were nontypeable and O128.

Comparison of the Wards and Banding Results

The comparison analysis between the hospital wards and the OMP banding types showed that 19.67% of the isolates from the OPD patients were AB type, 68.85% were BC type, and only 11.47% were ABC type. Among the hospitalized patients, 24% of the isolates were AB type, 66.66% were BC type, and 8.92%

Table 1: Frequencies and percentages of the banding patterns of the *E. coli* isolates collected from the patients in the different wards of Nemazee Hospital, Shiraz, Iran, 2012

Banding type'	Frequency (n)	Percentage	P value
OMP-I* (AB)	25	21.7	0.36
OMP-I (BC)	78	67.8	
OMP-α (ABC)	12	10.5	
Total	115	100	

*OMP-I type comprised AB and BC and ABC band was OMP-α

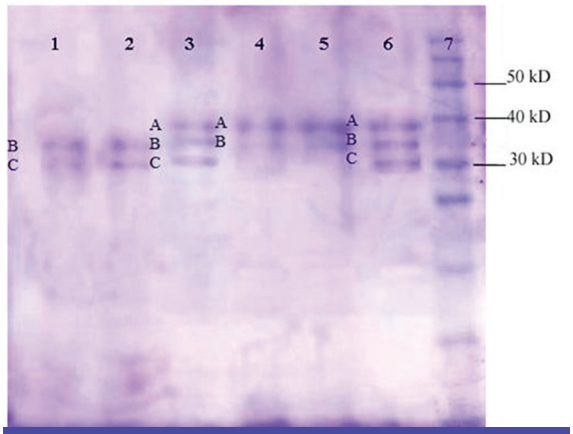

Figure 3: This is the SDS-PAGE analysis of the outer membrane protein profiles of the studied uropathogenic *E. coli* isolates. (Lanes 1 and 2 are BC banding patterns; lanes 3 and 6 are ABC banding type; lanes 4 and 5 are AB banding type, and lane7 is the marker protein).

were ABC type. No significant differences were detected in AB or BC banding types in the isolates between the hospitalized patients and the OPD ones. ABC type band pattern frequency in the isolates from the hospitalized patients was lower than that for the isolates from the OPD patients (P=0.008). The comparison analysis of the results is presented in figure 4.

In our study, no significant differences were detected in the frequencies of the SDS banding patterns of AB (P=0.23) and BC (P=0.26) between the isolates from the patients in the hospital wards and the isolates from the OPD patients.

Comparison of the Wards, Outer Membrane Protein Types, and Serological Tests of the E. coli Isolates

The comparison analysis between the wards, OMP types, and serotyping results showed that nontypeable BC type isolates were the most frequent isolates in the hospital wards and the OPD. AB with O114 and ABC with O44 were detected only in the hospital wards and ABC with O125, ABC with O86, ABC with O127, and BC with O44 were found only in the OPD isolates (figure 5).

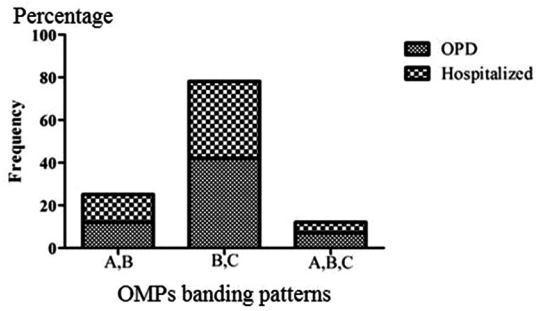

Figure 4: Comparisons are shown between the wards and the banding patterns of the studied uropathogenic *E. coli* isolates (P=0.12).

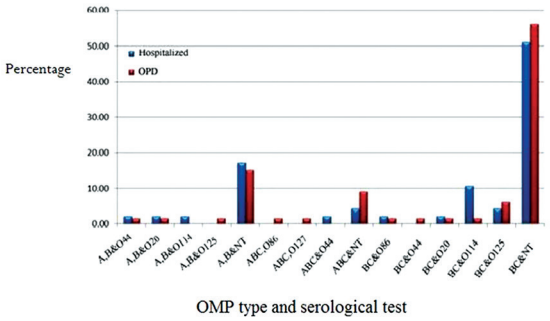

Figure 5: Comparisons between the wards, OMP types, and serological tests, showing the unequal distribution and high frequencies of the studied uropathogenic *E. coli* isolates with AB with nontypeable and BC with nontypeable OMP profiles in the hospital isolates compared with the OPD ones (NT: nontypeable).

Discussion

Although *E. coli* is carried in the intestinal tract as a harmless commensal, it is an important cause of UTI worldwide. The cell wall of *E. coli* as a Gram-negative bacterium contains the lipopolysaccharide outward of the outer membrane, which has special channels consisting of proteins called OMPs for the entry of hydrophilic molecules. A study of the OMPs in bacteria for both integral and lipid-linked membrane proteins is considered to be a practical way to determine their functional role in virulence and also the clonality or the heterogeneity of Gram-negative bacterial isolates.[15,18-20]

High copy number bacterial OMPs, including OmpA, OmpC, and OmpF, have been detected and characterized by using a variety of biochemical methods.[15,20-23] SDS-PAGE analysis of bacterial OMPs have shown to be a valuable method in the detection of various human and animal sources of infections in previous studies. Also, SDS-PAGE analysis of OMPs has been used to trace *E. coli* isolates from avian and calf infections.[14-16,20]

The present research is the first report of its kind to use both OMP patterns and O antigen to investigate the relationships between the collected human uropathogenic *E. coli* isolates. Our results revealed that the majority of the 115 samples collected from patients with UTI from Nemazee Hospital were from the OPD. The hospital isolates were mostly from the internal ward and the intensive care unit. The non-serologically typeable BC pattern of OMPs constituted the most frequent isolates in the hospital wards and the OPD. Isolates showing AB pattern with O114 antigen and ABC pattern with O44 antigen originated only from the hospital wards, whereas ABC types of OMPs with O86, O125, and O127 antigens and also BC type with O44 antigen were found only in the OPD isolates. Accordingly, BC type was the most prevalent OMP type of the 3 OMP types disseminated in either hospitalized or OPD patients widely. There is no human work on the OMP patterns of *E. coli* isolates. In agreement with our study, in an animal work on 23 *E. coli* isolates collected from calf diarrhea, SDS-PAGE showed the presence of proteins with 27 to 39 kDa molecular masses. It should be noted that the strains with O127 antigen and ABC type which were isolated in our OPD patients came from diarrheic calf in that study.[20] This might be due to the animal origin of *E. coli*, which may lead to UTI in OPD patients. Other O serotypes of *E. coli* isolates in our study might have been endogenous or hospital acquired.

Recent investigations in patients with UTI have shown different O serogroups of *E. coli* strains. In our study, we detected 9O serogroups of *E. coli*, with O125, O44, O128, O20, and O114 being the most common ones. Nonetheless, a study on the O serogroups of strains causing acute UTI in *E.coli* isolates from children with UTI in the Iranian city of Jahrom showed that the most common type of O antigen was O1 (12.2%).[24] A similar study in patients with UTI in the southeast of Iran determined that the most common types of O antigens were O2 (16.43%), O6 (16.43%), and O18 (13.69%).[25] Also, in a research on hospitalized or OPD patients with UTI in the emergency unit at Baqiyatallah Hospital in Tehran, Iran, 13 serogroups were determined (O1, O2, O6, O7,O16, O22, O75, O83, O4, O8, O15, O21, and O25)andO15 and O25 serogroups had the major prevalence.[26] In many studies in Iran,O6 is the most common type of uropathogenic *E. coli* found.[25] The difference might be related to the huge diversity of *E. coli* O serotypes inasmuch as more than 180 O antigens have been recognized for *E. coli*. The difference might also be reflected from the methods employed for O serotyping.

Overall, our results demonstrated an unequal distribution of the studied uropathogenic *E. coli* isolates in O serotypes in the hospital wards and the OPD, denoting a high diversity among *E. coli* strains detected via OMP pattern analysis using SDS-PAGE.

Given the prevalence of the BC type of OMPs and the role of OMPs in bacterial pathogenicity, it can be implied that there is a relationship between this specific OMP type and the ability of *E. coli* to cause outbreaks. Also, OMP typing for *E. coli* and many other bacteria can be useful for epidemiological studies and finding the source of infection.[16,27-30]

Similar to previous studies, the current research found no obvious relationship between a specific OMP types and *E. coli* serotypes. However, our comparative study between different hospital wards and the OPD and OMP types and serotypes showed that specific OMP types and serotypes were found only in the hospital wards. As a result, together with serotyping, OMP typing can help improve epidemiological studies.

A, B, and C bands were related to OmpF, OmpC, and OmpA, respectively. This determined a relationship between OMP banding, genetics, and bacterial virulence. Various functions of OMPs were determined, and a comparison analysis between the OMP bands and the function of each OMP was used to predict the bacterial virulence. The major bands in the current study were B and C, related to OmpC and OmpA. OmpA is a major target in host cell defense, and the beta-barrel structure of OmpA is important for outer membrane stability and involvement in bacterial virulence in *E. coli*. OmpC allows small molecular weight hydrophilic materials across the outer membrane and has antimicrobial resistance functions. Considering OMPs as a vaccine candidate against *E. coli* and suggesting future investigation in to any relationship between OMP type heterogeneity and virulence properties can be considered the practical applications of this study.

Conclusion

Our results demonstrated that of all the OMPs in the isolates collected from the hospitalized and OPD patients, OmpA and OmpC were the most prevalent proteins in the outer membrane of the studied uropathogenic *E. coli*. These results also showed a need for effective treatment strategy against OmpA and OmpC types of uropathogenic *E. coli* strains.

Acknowledgement

This study was financially supported by the Basic Sciences in Infectious Disease Research Center of Shiraz University of Medical Sciences (Grant #90-01-43-3250).

Conflict of Interest: None declared.

References

1. Yoshikawa TT. Antimicrobial resistance and aging: beginning of the end of the antibiotic era? J Am Geriatr Soc. 2002;50:S226-9. doi: 10.1046/j.1532-5415.50.7s.2.x. PubMed PMID: 12121517.
2. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev. 2011;24:141-73. doi: 10.1128/CMR.00027-10. PubMed PMID: 21233510; PubMed Central PMCID: PMC3021207.
3. Totsika M, Moriel DG, Idris A, Rogers BA, Wurpel DJ, Phan MD, et al. Uropathogenic Escherichia coli mediated urinary tract infection. Curr Drug Targets. 2012;13:1386-99. doi: 10.2174/138945012803530206. PubMed PMID: 22664092.
4. Buckles EL, Bahrani-Mougeot FK, Molina A, Lockatell CV, Johnson DE, Drachenberg CB, et al. Identification and characterization of a novel uropathogenic Escherichia coli-associated fimbrial gene cluster. Infect Immun. 2004;72:3890-901. doi: 10.1128/IAI.72.7.3890-3901.2004. PubMed PMID: 15213132; PubMed Central PMCID: PMC427398.
5. Saint S, Kowalski CP, Kaufman SR, Hofer TP, Kauffman CA, Olmsted RN, et al. Preventing hospital-acquired urinary tract infection in the United States: a national study. Clin Infect Dis. 2008;46:243-50. doi: 10.1086/524662. PubMed PMID: 18171256.
6. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2010;2:a000414. doi: 10.1101/cshperspect.a000414. PubMed PMID: 20452953; PubMed Central PMCID: PMC2857177.
7. Cress BF, Englaender JA, He W, Kasper D, Linhardt RJ, Koffas MA. Masquerading microbial pathogens: capsular polysaccharides mimic host-tissue molecules. FEMS Microbiol Rev. 2014;38:660-97. doi: 10.1111/1574-6976.12056. PubMed PMID: 24372337; PubMed Central PMCID: PMC4120193.
8. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67:593-656. doi: 10.1128/MMBR.67.4.593-656.2003. PubMed PMID: 14665678; PubMed Central PMCID: PMC309051.
9. Soltanmohammadi N [Internet]. Biochemical and biophysical characterization of various cell wall channel-forming proteins: IRC-Library, Information Resource Center der Jacobs University Bremen; 2013. Available from: https://www.deutsche-digitale-bibliothek.de/binary/PFWL6RAQJ4AO5V7QKPQDHAZKMGZTDHBI/full/1.pdf
10. Ricci DP, Silhavy TJ. The Bam machine: a molecular cooper. Biochim Biophys Acta. 2012;1818:1067-84. doi: 10.1016/j.bbamem.2011.08.020. PubMed PMID: 21893027; PubMed Central PMCID: PMC3253334.
11. Martorana AM, Motta S, Di Silvestre D, Falchi F, Deho G, Mauri P, et al. Dissecting Escherichia coli outer membrane biogenesis using differential proteomics. PLoS One. 2014;9:e100941. doi: 10.1371/journal.pone.0100941. PubMed PMID: 24967819; PubMed Central PMCID: PMC4072712.
12. Nash AA, Dalziel RG, Fitzgerald JR. Mims' pathogenesis of infectious disease. Amsterdam: Academic Press; 2000.
13. Polissi A, Sperandeo P. The lipopolysaccharide export pathway in Escherichia coli: structure, organization and regulated assembly of the Lpt machinery. Mar Drugs. 2014;12:1023-42. doi: 10.3390/md12021023. PubMed PMID: 24549203; PubMed Central PMCID: PMC3944529.
14. Tie Z, Chun-guang W, Xing-Hua Z. Clinical isolating outer membrane protein pattern from Avian Escherichia coli of China. Afr J Microbiol Res. 2010;4:1605-8.
15. Molloy MP, Herbert BR, Slade MB, Rabilloud T, Nouwens AS, Williams KL, et al. Proteomic analysis of the Escherichia coli outer membrane. Eur J Biochem. 2000;267:2871-81. doi: 10.1046/j.1432-1327.2000.01296.x. PubMed PMID: 10806384.
16. Kapur V, White DG, Wilson RA, Whittam TS. Outer membrane protein patterns mark clones of Escherichia coli O2 and O78 strains that cause avian septicemia. Infect Immun. 1992;60:1687-91. PubMed PMID: 1372298; PubMed Central PMCID: PMC257048.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75. PubMed PMID: 14907713.

18. Paino A [Internet]. Virulence properties of Aggregatibacter actinomycetemcomitans biofilm and characterisation of its putative cytokine exploitation. 2014. Avilable from: http://www.doria.fi/handle/10024/94128
19. NikaidoH.Multidrugresistanceinbacteria.Annu Rev Biochem. 2009;78:119-46. doi: 10.1146/annurev.biochem.78.082907.145923. PubMed PMID: 19231985; PubMed Central PMCID: PMC2839888.
20. Kandil MM, El-Said WG, Nagwa AS, Galal H, Marouf S, El-Jakee J, et al. Diversity of Escherichia coli Outer Membrane Protein. World Appl Sci J. 2011;15:1211-9.
21. Workman P, Heide K, Giuliano N, Lee N, Mar J, Vuong P, et al. Genetic, biochemical, and molecular characterization of the polypeptide transport-associated domain of Escherichia coli BamA. J Bacteriol. 2012;194:3512-21. doi: 10.1128/JB.06740-11. PubMed PMID: 22544271; PubMed Central PMCID: PMC3434720.
22. Masi M, Pages JM. Structure, Function and Regulation of Outer Membrane Proteins Involved in Drug Transport in Enterobactericeae: the OmpF/C . T o l C Case. Open Microbiol J. 2013;7:22-33. doi: 10.2174/1874285801307010022. PubMed PMID: 23569467; PubMed Central PMCID: PMC3617542.
23. Prehna G, Zhang G, Gong X, Duszyk M, Okon M, McIntosh LP, et al. A protein export pathway involving Escherichia coli porins. Structure. 2012;20:1154-66. doi: 10.1016/j.str.2012.04.014. PubMed PMID: 22658749.
24. Emamghorashi F, Farshad S, Kalani M, Rajabi S, Hoseini M. The prevalence of O serogroups of Escherichia coli strains causing acute urinary tract infection in children in Iran. Saudi J Kidney Dis Transpl. 2011;22:597-601. PubMed PMID: 21566331.
25. Rashki A, Abdi HA. O-serotyping of Escherichia coli Strains isolated from Patients With Urinary Tract Infection in Southeast of Iran. International Journal of Enteric Pathogens. 2014;2. doi: 10.17795/ijep20968.
26. Momtaz H, Karimian A, Madani M, Safarpoor Dehkordi F, Ranjbar R, Sarshar M, et al. Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob. 2013;12:8. doi: 10.1186/1476-0711-12-8. PubMed PMID: 23627669; PubMed Central PMCID: PMC3651382.
27. Luo D, Xue F, Ojcius DM, Zhao J, Mao Y, Li L, et al. Protein typing of major outer membrane lipoproteins from Chinese pathogenic Leptospira spp. and characterization of their immunogenicity. Vaccine. 2009;28:243-55. doi: 10.1016/j.vaccine.2009.09.089. PubMed PMID: 19796723.
28. Jay-Russell MT, Mandrell RE, Yuan J, Bates A, Manalac R, Mohle-Boetani J, et al. Using major outer membrane protein typing as an epidemiological tool to investigate outbreaks caused by milk-borne Campylobacter jejuni isolates in California. J Clin Microbiol. 2013;51:195-201. doi: 10.1128/JCM.01845-12. PubMed PMID: 23115263; PubMed Central PMCID: PMC3536209.
29. Nishimura LS, Ferreira LC, Pacheco AB, Guth BE. Relationship between outer membrane protein and lipopolysaccharide profiles and serotypes of enterotoxigenic Escherichia coli isolated in Brazil. FEMS Microbiol Lett. 1996;143:253-8. doi: 10.1111/j.1574-6968.1996.tb08489.x. PubMed PMID: 8837479.
30. Nishikawa Y, Helander A, Ogasawara J, Moyer NP, Hanaoka M, Hase A, et al. Epidemiology and properties of heat-stable enterotoxin-producing Escherichia coli serotype O169:H41. Epidemiol Infect. 1998;121:31-42. doi: 10.1017/S0950268898001046. PubMed PMID: 9747753; PubMed Central PMCID: PMC2809472.