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RESEARCH ARTICLE
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MOLECULAR CHARACTERIZATION AND COMPUTATIONAL STUDIES OF OMPC IN THE CLINICAL ISOLATES OF ESCHERICHIA COLI

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Abstract
Background/aim: Antimicrobial resistance (AMR) is a very severe threat and a major public health issue. Outer Membrane Porin C (OmpC) is an outer membrane protein in bacteria, associated with the modulation of cellular permeability and antibiotic resistance. In this study a clinical strain of Escherichia coli was used to screen for OmpC.

Materials and methods: Genomic DNA of the strain was extracted and screened for the presence of porins by designing specific primers and amplifying the coding gene using conventional PCR. After amplification, PCR product (OmpC gene) was sequenced through Sanger Sequencing method. OmpC of E. coli ATCC and CI were also analyzed by using different computational tools. BLAST and Clustal Omega were used to analyze nucleotide and protein alignment.

Results: Protein alignment result showed various mutations i.e P11S, D47N, V50E, S88N, Q173K, S178D, G233R, L234Y, I235L, T237N, V312I, A358D etc in OmpC amino acid residues of *E. coli* CI.

Conclusion: Hence, this study determines that the structure as well as the function of OmpC can be altered due to various mutations.
Key words: OmpC, Computational, In-silico, Antibiotic Resistance

1. Introduction

Antibiotic either inhibits bacteria cell function by primary drug-target interaction or induce cell death. Resistance against antibiotics is normally developed in microorganism due to evolutionary process

and by misuse of antibacterial drugs. Number of mechanisms are involved to develop resistance in pathogens [Dörr et al., 2010]. It is essential to minimize the resistance development and preserve the efficacy of existing drugs. Improving sanitation and controlled measures are necessary for prevention. Today for both science and medicine, the spread of antibiotic resistance is a unique challenge.

Antimicrobial activity and pathogenic nature of microbes alter due to change in composition of outer membrane. Bacterial outer membrane consists up of phospholipids, lipopolysaccharides (LPS), lipoproteins, and β-barrel porins (Henderson et al., 2016). These are involved in various functions such as biofilm synthesis, active transport of component linked with inner membrane (Bunik et al., 1997). They also provide defence mechanism against antibiotics (Nikaido and Vaara, 1985). The porins are the most abundant proteins of the outer membrane in Gram-negative bacteria, with various existing types. They can be classified as non-specific or specific porins based on their activity. Additionally, they are classified into monomeric, dimeric, or trimeric porins based on their functional structure (Koebnik et al., 2000; Pages et al., 2008). Outer Membrane Proteins such as surface antigens act as receptors for colicins and bacteriophage, involved to induce immunity in host cell (Miller et a., 1998). Antibiotic susceptibility is interlinked with down and up regulation of OMPs. For example bacteriophage, involved in resistant against carbapenems due to OprD protein loss from OMs. Loss of OmpC and OmpF cause resistance in Escherichia coli and Klebsiella pneumoniae against beta-lactams (Mushtaq et al., 2008). Neisseria gonorrhoeae resistant to penicillin and tetracycline due to mutation in por(IB) gene (Olesky et al., 2002). OmpC plays a major role in antibiotic transport and the maintenance of membrane integrity. This study is designed to determine various mutations altering structure as well as the function of OmpC.

2. Materials and methods

2.1. Study design and sampling

In this study clinical isolates (CI) of *E. coli* already available in the stocks of SA-CIRBS, IIU, Islamabad-Pakistan lab were used. Quality control (QC) strains of American Type Culture Collection (ATCC), *E. coli* (25922, ESBL-negative) were used to ensure the quality of bacterial cultures and in vitro antibacterial sensitivity testing following Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020).

Fresh cultures of both strains were prepared by picking up some from CI and ATCC cultures and streaking these on autoclaved agar media plates and incubating overnight at 37˚C.

2.2. Antimicrobial Susceptibility detection:

Antimicrobial susceptibility testing for different types of antibiotics such as Ciprofloxacin (5µg), Ampicillin (10µg), Erythromycin (15µg), Kanamycin (30µg) and Tetracycline (30µg) was performed for all the isolates by Kirby–Bauer disk-diffusion method according to CLSI guidelines 2020 (CLSI, 2020).

2.3. DNA Extraction

For DNA Extracton, the well-isolated colonies of *E.Coli* were picked up with a sterile loop and inoculated in nutrient broth. These cells were incubated in shaking incubator for maximum growth. Genomic DNA was isolated from the bacterial cultures WizPrep™ gDNA Mini Kit by following the instructions. Gel Electrophoresis of extracted DNA was performed and band image were visualized on the computer screen by using image lab software.

2.4. Primers Designing

Primers were designed by using Snap Gene tool to amplify OmpC (1.1kb) of *E. coli* for PCR reaction. The forward primer (5′-ATGAAAGTTAAAGTACTGTCCCTCCTGG-3′) was 28 nucleotides long, with 42.86% GC content whereas, reverse primer (5′-TTAGAACTGGTAAACCAGACCCAGAG -

3′) was 26 nucleotides long with 46.15% GC content. Both primers have temperature 67.2 ˚C and 66.3 ˚C respectively.

2.5. Polymerase Chain Reaction:

To perform the PCR amplification of target regions, PCR reaction mixture was prepared with a total volume of 50 µL containing:10µl Master Mix (1X) (Solis Biodyne), 18 µl PCR grade water (BIO Basic WW 1002), 10µl of each primer (1 µM) and 2 µl genomic DNA. Standard PCR reaction was performed in a thermal cycler™ with following reaction conditions: Initial denaturation at 95 °C for 3 min followed by 30 cycles of denaturation (95 °C for 30s), Primer annealing (63 °C for 30s), elongation (72 °C for 2min) and final incubation at 72 °C for 7min. The reaction was cooled at 10°C for 10 min.

Electrophoresis was performed to validate PCR reaction. 5 µl of the final reactions were loaded on the 1.5% agarose gel wells with a reference DNA ladder of appropriate concentration and size. The gel was run for 1 hour and was visualized under Gel Doc System after staining with 0.5 µg ethidium bromide solution for 10 min. A band on the gel confirms successful amplification of the gene. DNA purification kit (SolarBio Cat#D1300) was used to purify samples. The purified sample was sent to Korea (Macrogen) for Sanger sequencing.

2.6. In silico study of E. coli OmpC protein:

Single Nucleotide Polymorphism (SNP) in OmpC gene is associated with resistance in clinical isolate of *E. coli*. Different computational tools were used in this study for SNPs identification which has deleterious effect on structure and function of OmpC protein such as Expasy, BLAST, Clustral Omega (for nucleotide and protein alignment), Phyre2, UCSFChimera etc.

a. **ExPASy:** Expert Protein Analysis System (ExPASy) was used for translation of nucleotide sequence.
b. **BLAST-P and Clustal Omega:** BLAST-P and Clustal Omega online tools were used for multiple sequence alignment. All these tools work on pairwise global alignment method.
c. **PredictProtein tool:** It was used for structural annotation as well as for functional annotation of protein.
d. **Phyre2:** This online data tool was used to predict the 3D structure of OmpC of *E. coli*.
e. **UCSF Chimera:** This tool was used to visualize structure of resultant protein and to highlight the point mutations.

2.7. Statistical analysis

Microsoft excel was used to perform the data analysis.

3. Results

3.1. Antibiotic susceptibility essay:

Both clinical isolate and ATCC strains of *Escherichia coli* were treated for antimicrobial susceptibility test using disk diffusion method. Ciprofloxacin, Tetracycline and Erythromycin showed activity against *E. coli* clinical isolate. *E. coli* CI was highly resistant against two antibiotic discs i.e Ampicillin and Kanamycin. However, *E. coli* ATCC showed activity against all of these antibiotic discs except ampicillin (Figure 1).

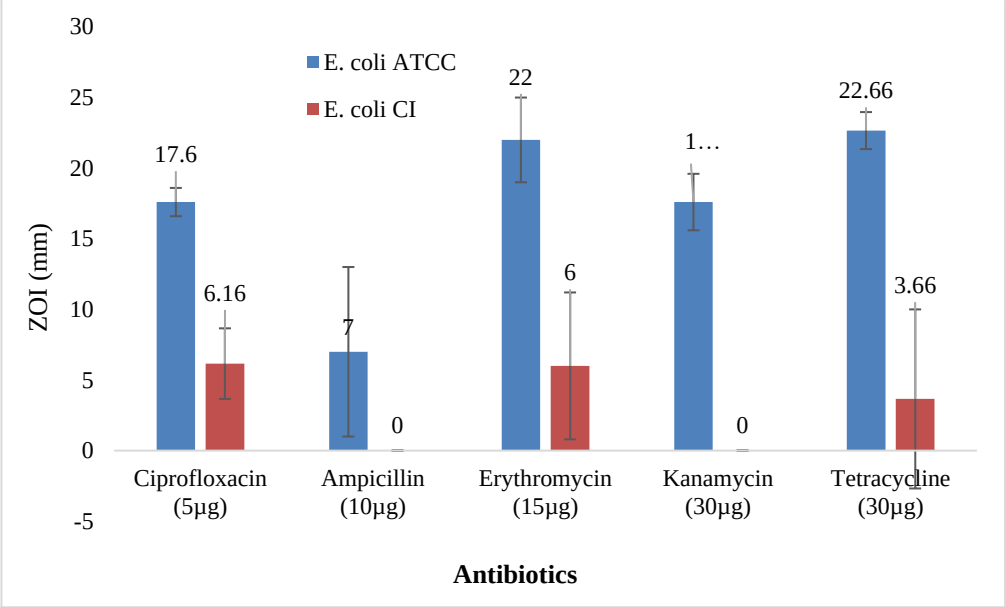

Figure 1: Antibiotics susceptibility of *E. coli* ATCC & CI against selected antibiotics which shows that E.Coli CI has highest resistance against Kanarmycin and ampicillin, and lowest against Ciprofloxacin.

3.2. DNA extraction:

Micro plate reader was used for quantitative analysis of DNA. Extracted DNA concentration was 152µg/ml with 1.91 ratios at 260/280.

3.3. Polymerase Chain Reaction:

The basic purpose of this study is to analyze the outer membrane porin which is involved in developing resistance in clinical isolate of *E. coli*. By using PCR, DNA fragment can be isolated from *E. coli* clinical isolate by specific amplification of DNA region. OmpC gene was isolated from *E. coli* CI genomic DNA by using PCR. For PCR product analysis gel electrophoresis was used as shown in Figure 2. By comparing with DNA ladder, fragment size can be find out. DNA fragment of 1128bp (OmpC) was separated on 1% agarose gel.

Figure 2: Outer membrane porin C band visualized by agarose gel electrophoresis.

3.4. Protein alignment results:

4. The BLAST-P and Clustal Omega online tools were used for multiple sequence alignment. All these tools work on pairwise global alignment method. Results are shown in Figure 3 and 4. Clustal omega results for protein alignment are shown in figure 4. We can see changes in the protein sequence of CI strain as compared to ATCC strain. The details are also given in table 2.

Figure 3: Description and graphic result of protein BLAST

Figure 4: Protein alignment results showing comparison between OmpC of ATCC strain and CI.

4.1. PredictProtein tool Results:

PredictProtein tool was used for structural annotation as well as for functional annotation of protein as shown in Table 1.

Table 1. Structure & function analysis of E. coli ATCC & CI OmpC protein

	Annotation of protein structure and function	***E. coli* ATCC**	***E. coli* CI**
Structural annotation	Sequence length	375	367
	Number of aligned protein	41	38
Molecular function ontology	Transport activity	56% reliability	54% reliability
	Transmembrane transport activity	56% reliability	54% reliability
Biological process ontology	Transport	61% reliability	60% reliability
	Ion transport	33% reliability	32% reliability

4.2. OmpC Protein 3D structure Prediction:

The Phyre2 online tool was used to design Monomer 3D structure of OmpC and shown in Figure 5.

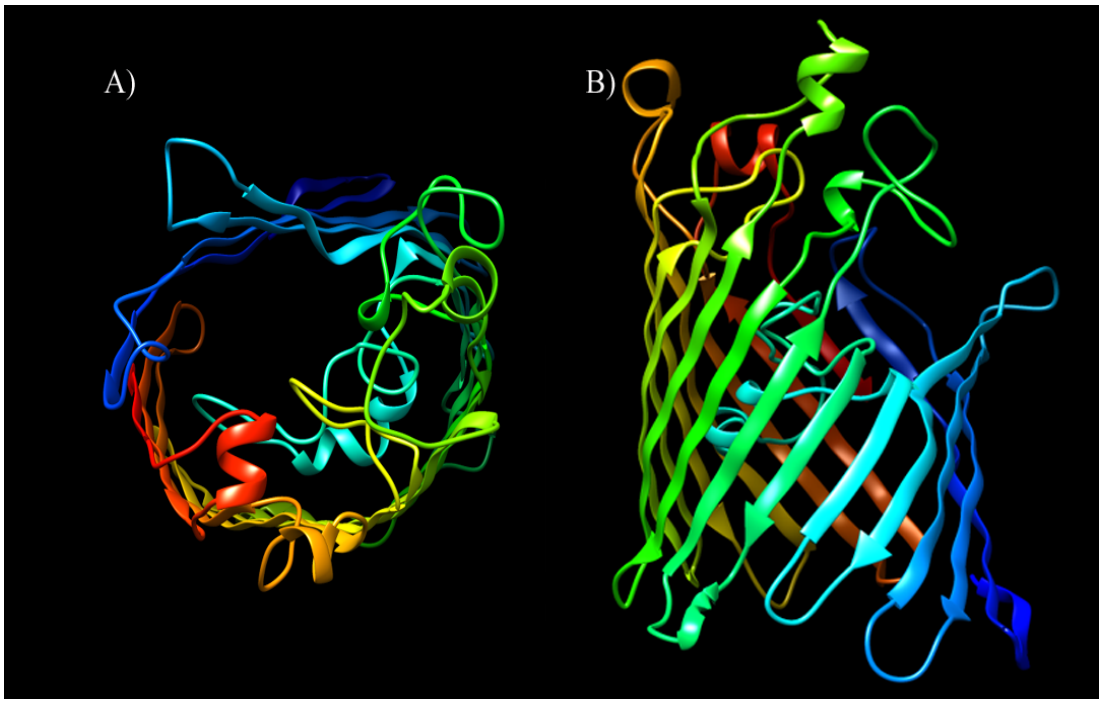

Figure 5. The structure of the OmpC monomer. (A) Viewed from the side, parallel to the membrane. (B) The structure view from top, perpendicular to the membrane.

4.3. Distribution of Amino Acid Substitutions in OmpC

The differential distribution of point mutations was analyzed (Table 2) and the mutation sites were highlighted, and amino acid sequences were tagged using Chimera software (Figure 6).

Table 2. Change in amino acids residues between OmpC of ATCC & CI

Serial No.	Change in amino	Serial No.	Change in amino acid
1	Pro11Ser	14	Val210 Ile
2	Asp47Asn	15	Trp 221D
3	Val50Glu	16	Asn225Phe
4	Ala85Ser	17	Thr 232Glu
5	Pro86Ala	18	Gly233Arg
6	Ser88Asn	19	Leu234Tyr
7	Ile106 Val	20	Ile235Leu
8	Gln169Ser	21	Thr237Asn
9	Gln173Lys	22	Val321Ile
10	Ser178Asp	23	Ala322Asn
11	His180Glu	24	Gln 354Arg
12	Ile182Met	25	Ala354Asp
13	Lys188Gly		

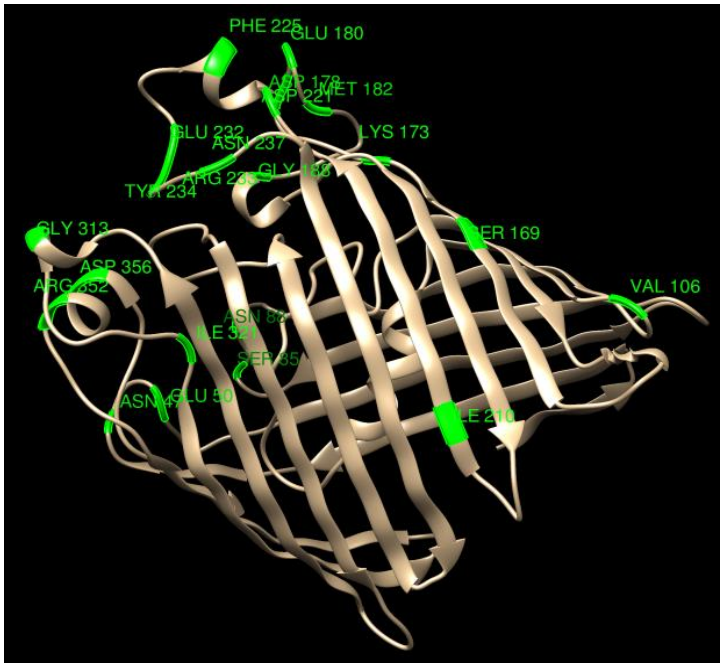

Figure 6. Distribution of Amino Acid Substitutions in 3D structure of OmpC. Monomer representations of the OmpC is highligjhted and tagged for amino acid positions related to point mutations. OmpC of CI isolate in comparison to ATCC isolate has point mutations at 25 sites e.g from Aspartate to Asparagine at position 47, Valine to Glutamic acid at position 50, Isoleucine to Methionine at position 182, Alanine to Aspartate at position 358 and many more.

5. Discussion

This research was planned to determine molecular identification of porin coding gene in clinical isolate of *E. coli*. Results of susceptibility test coincides with previous studies that *E. coli* clinical isolate show resistance for Ampicillin and Ciprofloxacin (Uma et al., 2009; Ali et al., 2010). DNA extraction was done and DNA concentration was measured. After amplification of OmpC, results were analyzed by using agarose gel electrophoresis. In qualitative analysis, size of OmpC was determined by comparing with DNA ladder i.e about 1100bps. After amplification of OmpC, PCR product was sequenced by using Sanger sequencing method. Mutations in OmpC of clinical isolate was determined by using in silico study. For computational analysis of OmpC different tools were used such as Clustal Omega, BLAST, Expasy, Prot Param, Pyre2 etc. Expasy was used for translation of nucleotide sequence.

Clustal Omega and BLAST tools were used for protein alignment. The blast results were more significant with 0.0 E-vale (expect value) and 89.89% identity with *E. coli* 25922 (ATCC strain). By comparing protein sequence of *E. coli* 25922 with CI, various mutations were determined in residues position as shown in Table 1. These mutations may decrease the stability of outer membrane protein. Previous studies revealed that due to decreased in protein stability cause misfolding, aggregation and degradation of protein structure and function (Du et al., 2005). These amino acids are involved in protein interaction. So, these mutations are considered as damaging to structure and function of OMP. To determine the mutation altering the structure of OmpC protein, Phyre2 tool was used to generate the structure of OmpC protein. Phyre2 use d1osma Template to predict protein structure and chimera was used to visualize that 3D model of protein. This model showed slight variation with *E. coli* ATCC strain. Previous studies suggested that OmpC play an important role in the transport of various antibiotics such as β-lactams. OmpC deletion increases the MIC of various antibiotics. Furthermore, it's also involved to adapt envelop for stress (Choi and Lee, 2019). OMP alteration plays an important role in resistance development against various antibiotics, but various reports describe the diminished expression of porins in clinical isolates (Delcour, 2009).

Conclusion:

The basic purpose of the research is molecular characterization of genes that code porin in *E. coli* CI. Protein alignment result shows various mutations i.e D47N, V50E, S88N, Q173K, S178D, H188E, A313N, I190M, K196G, W229D, G235R, L236Y, I237L, T239N, V312I, A358D in amino acid residues of *E. coli* CI OmpC. ProtParam was used to determine the physicochemical properties of OmpC. These properties play very important role to determine the permeability of outer membrane. OmpC involves in transport of various β-lactams antibiotics. Antibiotic penetration in clinical isolates limited due to variation in structure of protein. Hence, this study determines that the structure as well as the function of OmpC can be altered due to various mutations.

References

1. Ali SQ, Zehra A, Naqvi BS, Shah S, Bushra R (2010). Resistance pattern of ciprofloxacin against different pathogens. Oman Medical Journal 25(4):294-298. doi:10.5001/omj.2010.85
2. Bunik V Shoubnikova A Bisswanger H Follmann H (1997). Characterization of thioredoxins by sodium dodecyl sulfate-slab gel electrophoresis and high performance capillary electrophoresis. Electrophoresis 18(5):762-766. Doi: 10.1002/elps.1150180517
3. Choi U, Lee CR (2019). Distinct Roles of Outer Membrane Porins in Antibiotic Resistance and Membrane Integrity in Escherichia coli. Frontiers in Microbiology 10:953. doi:10.3389/fmicb.2019.00953
4. Clinical and Laboratory Standards Institute (2020). *Performance Standards* for *Antimicrobial Susceptibility Testing*. 30th ed. *CLSI supplement M100*. Wayne, PA: *Clinical* and *Laboratory Standards Institute*
5. Delcour AH (2009). Outer membrane permeability and antibiotic resistance. Biochimica et Biophysica Acta 1794(5):808-816. doi:10.1016/j.bbapap.2008.11.005
6. Dörr T, Vulić M, Lewis K (2010). Ciprofloxacin causes persister formation by inducing the TisB toxin in Escherichia coli. PLoS Biology 8(2):e1000317. doi:10.1371/journal.pbio.1000317
7. Du K, Sharma M, Lukacs G (2005). The ΔF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nature Structural & Molecular Biology 12:17–25. https://doi.org/10.1038/nsmb882
8. Henderson JC, Zimmerman SM, Crofts AA, Boll JM, Kuhns LG et al. (2016). The Power of Asymmetry: Architecture and Assembly of the Gram-Negative Outer Membrane Lipid Bilayer. Annual Review of Microbiology 70:255-78. doi: 10.1146/annurev-micro-102215-095308
9. Koebnik R, Locher KP, Van Gelder P (2000). Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Molecular Microbiology 37,239–253. doi: 10.1046/j.1365-2958.2000.01983.x
10. Miller J, Williamson ED, Lakey JH, Pearce MJ, Jones SM et al. (1998). Macromolecular organisation of recombinant *Yersinia pestis* F1 antigen and the effect of structure on immunogenicity. *FEMS Immunology & Medical Microbiology* 21(3)213–221. https://doi.org/10.1111/j.1574-695X.1998.tb01168.x
11. Mushtaq S, Ge Y, Livermore DM (2004). Comparative activities of doripenem versus isolates, mutants, and transconjugants of Enterobacteriaceae and Acinetobacter spp. with characterized beta-lactamases. Antimicrobial Agents and Chemotherapy 48(4):1313-1319. doi:10.1128/AAC.48.4.1313-1319.2004
12. Nikaido H, Vaara M (1985). Molecular basis of bacterial outer membrane permeability. Microbiological Reviews 49(1), 1–32. https://doi.org/10.1128/mr.49.1.1-32.1985
13. Olesky M, Hobbs, M, Nicholas, RA (2002). Identification and analysis of amino acid mutations in porin IB that mediate intermediate-level resistance to penicillin and tetracycline in Neisseria gonorrhoeae. Antimicrobial agents and Chemotherapy, 46(9), 2811–2820. https://doi.org/10.1128/AAC.46.9.2811-2820.2002

14. Pages JM, James, CE, Winterhalter M (2008). The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nature Reviews Microbiology 6:893–903. doi: 10.1038/nrmicro1994
15. Uma B, Prabhakar K, Rajendran S, Kavitha K, Sarayu YL (2009). Antibiotic Sensitivity and Plasmid Profiles of Escherichia coli Isolated from Pediatric Diarrhea. Journal of Global Infectious Diseases 1(2):107-110. doi:10.4103/0974-777X.56255