Original Article

From Bench to Bedside: A Comprehensive Study on Pardaxin Peptide's Antimicrobial Effect on *Escherichia coli*, Including Clinical Isolates

Aminnia, P[1], Sharifi Niknafs, A[1*], Doustdar, F[1]

1. *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ABSTRACT

Escherichia coli is a common cause of urinary tract infections and has shown increasing resistance to available antimicrobial agents. Antimicrobial peptides, such as Pardaxin, offer a potential alternative to traditional antibiotics due to their ability to disrupt bacterial cell membranes through interaction with the lipid bilayer. This mode of action reduces the likelihood of resistance development compared to conventional antibiotics that target specific cellular processes. The objective of this study was to assess the antimicrobial efficacy of the Pardaxin peptide against both standard and clinical strains of *E. coli*. *E. coli* ATCC 25922 was used as the standard strain, and 20 samples derived from patients were included in the study. Isolation and identification of *E. coli* were performed using enrichment media, selective media, and biochemical tests. Bacterial cultures were conducted on Mueller-Hinton agar, and the antimicrobial effect of the Pardaxin peptide was assessed using classic disk diffusion tests. During the disk diffusion test, a distinct area of no growth was observed surrounding the Pardaxin disks for both the standard and clinical strains. In the microdilution test, the minimum inhibitory concentration (MIC) of Pardaxin was found to be 390 µg/ml for the clinical strain and 450 µg/ml for the standard strain. These concentrations are comparable to the 500 µg/ml concentration of erythromycin, indicating the antibacterial properties of Pardaxin against **E. coli**. The results of this study provide evidence for the antimicrobial properties of the Pardaxin peptide against both standard and clinical strains of *E. coli*.

Keywords: Escherichia coli; Antimicrobial Peptides; Pardaxin Peptide; Microbial resistance; Minimum Inhibitory Concentration

1. Introduction

Escherichia coli (E. coli) is a Gram-negative bacterium commonly found in the intestines of humans and animals. While most strains of *E. coli* are harmless, some can cause serious infections, including food poisoning, urinary tract infections, and pneumonia. *E. coli* is also a major contributor to antibiotic resistance, which poses a serious global health problem (1). The increasing resistance of E. coli to available antimicrobial agents presents significant challenges in managing these infections (2). In response to the emergence of drug-resistant variants, there is a growing interest in alternative antimicrobial agents that can effectively target *E. coli* without fostering resistance (3). Antimicrobial peptides (AMPs) have gained significant attention as potential alternatives to traditional antibiotics (4). AMPs are oligopeptides that play a crucial role in the innate immune response against a wide range of pathogens, including bacteria, viruses, and fungi (5). One AMP that has demonstrated promising antimicrobial activity against various pathogens is Pardaxin. Pardaxin is a 33-amino acid peptide originally isolated from the skin mucus of the Red Sea Moses sole fish. It has been extensively studied for its antimicrobial properties and has shown efficacy against both Gram-positive and Gram-negative bacteria (6, 7). The mechanism of action of Pardaxin involves the disruption of the bacterial cell membrane. Pardaxin interacts with the lipid bilayer of the cell membrane, leading to pore formation and subsequent leakage of intracellular contents (8, 9). This mode of action is particularly advantageous as it reduces the likelihood of resistance development compared to traditional antibiotics that target specific cellular processes (8, 10). Given the unique mechanism of action of the Pardaxin peptide and the increasing resistance of *E. coli* to available antimicrobial agents, we aimed to evaluate the antimicrobial effect of Pardaxin on both standard and clinical variants of *E. coli*.

2. Materials and Methods

The standard strain of **E. coli** used in this study was ATCC 25922. Additionally, 20 clinical isolates of *E. coli* were collected from patients with urinary tract infections (UTIs), with one isolate selected per patient prior to antibiotic therapy. Pardaxin peptide was purchased from MIMOTOPES, Australia, in pure form for further experimentation.

2.1. Characterization of *E. coli* Strains

E. coli strains were initially isolated on MacConkey agar. Biochemical tests, including the Indole, Methyl Red, Voges-Proskauer, Citrate, Triple Sugar Iron (TSI), Hydrogen Sulfide (H2S), Urease, Lysine Decarboxylase (LDC), Gas Production, and Sulfide Indole Motility (SIM) tests, were used to determine their biochemical properties. These tests assessed various aspects, such as indole production, acid production during glucose fermentation, acetoin production, citrate utilization, fermentation patterns, hydrogen sulfide production, urea hydrolysis, lysine decarboxylase activity, gas production, and motility. Gram staining confirmed their Gram-negative nature.

2.2. Bacterial Culture

MacConkey agar was used as the culture medium for growing *E. coli* strains. Agar plates were prepared according to standard protocols. Bacterial strains were streaked onto the agar plates and incubated at 37ºC for 24 hours. Antimicrobial Susceptibility Testing. The antimicrobial effect of the Pardaxin peptide on *E. coli* strains was determined according to CLSI guidelines using the standard Kirby-Bauer disk diffusion method and microdilution broth assays (11). Commercially prepared antibiotic disks used in the disk diffusion test included cotrimoxazole, amikacin, gentamicin, nalidixic acid, nitrofurantoin, imipenem, and ceftazidime. Each antibiotic disk was placed on 150 mm Mueller-Hinton agar plates inoculated with *E. coli* strains, and the plates were incubated at 37ºC for 16-20 hours. Following the incubation period, we prepared a bacterial suspension in physiological saline with a turbidity equivalent to a 0.5 McFarland standard, approximating a bacterial count of 10^8 CFU/mL. The diameters of the clear zones of inhibition were measured using precision calipers and recorded as indicators of antimicrobial activity.

2.3. Minimum Inhibitory Concentration (MIC) Determination

The MIC values of Pardaxin peptide and erythromycin were determined for both the standard *E. coli* strain (ATCC 25922) and the clinical *E. coli* samples. MIC assays were performed using the microdilution method. Serial dilutions of the peptides were prepared in appropriate growth media, and bacterial strains were inoculated into each dilution. The lowest concentration of the peptide that inhibited visible bacterial growth was recorded as the MIC.

3. Results

3.1. Characterization of *E. coli* Strains

The *E. coli* strains exhibited positive results for the Indole and Methyl Red tests, indicating their ability to produce indole and the presence of acid production from glucose metabolism. Conversely, they yielded negative results for the Voges-Proskauer (VP) and Citrate tests, suggesting the absence of acetoin production and the inability to utilize citrate as a carbon source. In the TSI medium, the isolates demonstrated an A/A reaction, indicating both acid and gas production. They also tested negative for hydrogen sulfide (H2S) production and urease activity but positive for lysine decarboxylase (LDC) activity. Additionally, they exhibited motility in the SIM medium. Furthermore, all E. coli strains exhibited characteristic Gram-negative staining, confirming their classification. Disk Diffusion Results of Antibiogram. The antimicrobial susceptibility profiles of *E. coli* isolates were determined via the Kirby-Bauer disk diffusion technique. Inhibition zone diameters were quantified and categorized as indicating susceptibility, intermediate susceptibility, or resistance according to the 2018 Clinical

and Laboratory Standards Institute (CLSI) guidelines. The susceptibility profile is presented in Table 1.

3.2. Minimum Inhibitory Concentration (MIC) Determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the Pardaxin peptide and erythromycin were determined for the standard *E. coli* strain (ATCC 25922) as well as for the clinical *E. coli* samples. The results indicated that Pardaxin exhibited a lower MIC compared to erythromycin for both the standard and clinical *E. coli* strains. Detailed MIC and MBC values are presented in Table 2.

4. Discussion

Antimicrobial peptides (AMPs) are a class of antimicrobial agents that have garnered significant attention due to the increasing resistance of pathogenic bacteria to traditional antibiotics. This study aimed to evaluate the antimicrobial effect of the Pardaxin peptide on both standard and clinical variants of *E. coli*, highlighting its potential as an effective antimicrobial agent. In a cross-sectional study conducted by Rajabnia et al., the prevalence and antibiotic resistance patterns of ESBL-producing *E. coli* isolated from patients with urinary tract infections (UTIs) were assessed. Out of 291 *E. coli* isolates, 108 (37.11%) were identified as ESBL producers, while 183 (62.89%) were non-ESBL producers. Among the ESBL-producing *E. coli*, the highest antibiotic resistance was observed against cefotaxime, amoxicillin, and piperacillin, whereas the highest sensitivity was noted for meropenem, nitrofurantoin, and gentamicin. These findings suggest that cephalosporins, penicillins, and

cotrimoxazole are not recommended for the treatment of ESBL-producing *E. coli*, while carbapenems and aminoglycosides are suggested as effective treatment options (12). The disk diffusion results of our study revealed the susceptibility patterns of the *E. coli* strains to a panel of antibiotics. Notably, resistance to commonly used antibiotics, such as cotrimoxazole and gentamicin, was observed, consistent with the global trend of increasing antibiotic resistance in *E. coli* (13). Previous studies have investigated the identification of other potential antibiotics. ClpP protease inhibitors, specifically α-amino diphenyl phosphonates, represent an exciting development (14). Additionally, peptide nucleic acid (PNA) has emerged as a promising treatment option, effectively reducing colistin resistance in *E. coli* by inhibiting the expression of the mcr-1 gene (15). Our study demonstrated the antimicrobial properties of the Pardaxin peptide against both standard and clinical strains of *E. coli*, suggesting its potential as an alternative treatment. The MIC determination of the Pardaxin peptide demonstrated its efficacy as an antimicrobial agent against both the standard *E. coli* strain and the clinical isolates. The peptide exhibited a lower MIC against the clinical strain compared to the standard strains, indicating potential variations in susceptibility among different *E. coli* populations. These findings support the notion that the antimicrobial effect of the Pardaxin peptide may be influenced by the genetic diversity and adaptive mechanisms of *E. coli* strains (16). The results of this study contribute to the growing body of evidence highlighting the potential of antimicrobial peptides as alternatives to traditional antibiotics. AMPs, such as Pardaxin, offer

Table 1. Disk diffusion results of antibiogram for *E. coli* strains

Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Cotrimoxazole	7 (35)	1 (5)	12 (60)
Amikacin	14 (70)	1 (5)	5 (20)
Gentamicin	3 (15)	1 (5)	16 (80)
Nalidixic acid	7 (35)	0 (0)	13 (65)
Nitrofurantoin	7 (35)	2 (10)	11 (55)
Imipenem	17 (85)	2 (10)	1 (5)
Ceftazidime	10 (50)	1 (5)	9 (45)
Total	**46.43 %**	**5.71 %**	**47.16 %**

Table 2. MIC determination for Pardaxin peptide and erythromycin

	Pardaxin		Erythromycin	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
Standard *E. coli* (ATCC 25922)	450	320	500	450
Clinical *E. coli* Strains	390	300	500	450

MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

several advantages, including broad-spectrum activity, a low likelihood of resistance development, and potential effects on bacterial membrane integrity (8, 10, 16). The disruption of the bacterial cell membrane by the Pardaxin peptide can be an effective mechanism for combating *E. coli* infections, particularly given the susceptibility of this pathogen due to its unique outer membrane structure (8). In conclusion, this study provides valuable insights into the antimicrobial effects of the Pardaxin peptide on *E. coli* strains. The findings suggest that Pardaxin holds promise as a novel antimicrobial agent against *E. coli*, particularly in the context of UTIs. However, several limitations should be acknowledged. This study focused solely on the antimicrobial effect of the Pardaxin peptide on *E. coli* strains. Further research is needed to explore the potential synergistic effects of Pardaxin with other antimicrobial agents, and additional development efforts are warranted to harness the full potential of the Pardaxin peptide and investigate its clinical applications in combating *E. coli* infections.

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Authors' Contribution

PA, ASN, and FD conceptualized the study. PA and ASN Performed the investigation. ASN and PA drafted and ASN and FD revised the paper. All authors read and approved that the final manuscript complied with the relevant Instructions to Authors, the Ethics in Publishing policy, and Conflicts of Interest disclosure.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare no conflicts of interest.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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