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Wael Adil Obaid

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Prevalence and characterization of uropathogenic virulence genes in *Staphylococcus saprophyticus* isolated from women with urinary tract infections in Baghdad, Iraq

Wael Adil Obaid
Department of Applied Pathological Analysis, College of Science, Al-Nahrain University, Jadiriya, Baghdad, Iraq

Urinary tract infections (UTIs) are a considerable global concern and are among the most prevalent types of microbial infection globally. Young and sexually active women are disproportionately affected, with over 60% of females being diagnosed with UTIs at some point in their lives. *Staphylococcus saprophyticus* is a significant pathogen that causes UTIs in the community. The pathophysiology of coagulase-negative staphylococcal UTIs relies significantly on virulence factors and their expression. The main goal of the current investigation was to ascertain the occurrence of uropathogenic genes in *S. saprophyticus* isolated from urine samples of women who had suspected UTIs in Baghdad hospitals in Baghdad, Iraq. The prevalence of *S. saprophyticus* in urine samples was identified with the microscopic patterns, cultural and biochemical characteristics, Vitek 2 Compact System and *S. saprophyticus*-specific primers. Nine virulence genes, including *Ssp, UafA, Aas, sarA, sdrI, rot, dsdA, capD* and *agr* were screened using multiplex polymerase chain reaction. Out of the 300 samples, 31.66% harbored *S. saprophyticus* pathogens, confirmed by the existence of *S. saprophyticus-specific* primers in all isolates. The current data indicated that all isolates tested positive for the *UafA, sarA, rot,* and *agr* genes. Moreover, the results revealed that 94.74% of isolates tested carried the *Aas* gene, followed by *Ssp* (84.2%) and lastly *dsdA* (20%). However, *SdrI* and *CapD genes* were not detected in any of the isolates. These findings demonstrate that *S. saprophyticus* is a major contributor to UTIs in women in this specific region with the prevalence of certain virulence genes for this bacterium.

Keywords: Urinary Tract Infection, *Staphylococcus saprophyticus*, Isolates Women, Uropathogenic Potential, Virulence Genes

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CORRESPONDENCE TO
Wael Adil Obaid,
Department of Applied Pathological Analysis, College of Science, Al-Nahrain University, Jadiriya, Baghdad, Iraq
Email: Waeladil@nahrainuniv.edu.iq
https://orcid.org/0009-0007-8649-8370
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INTRODUCTION

Urinary tract infections (UTIs) are highly prevalent globally, contributing significantly to mortality, morbidity and economic burden (Yang *et al*., 2022). UTIs are notably prevalent among sexually active young women, presenting effective preventive strategies and posing a substantial challenge in healthcare management (Seid *et al*., 2023; Khan *et al*., 2023). Women have a higher vulnerability to UTIs due to structural and physiological traits of their urethra (Chua *et al*., 2023). At least one UTI affects over 60% of women in their lifetime, as well as 20-30% of those women will experience recurrent infection in the next six months (Medina and Castillo-Pino, 2019).

The incidence of UTI rises in correlation with age, particularly in women over 65 years. This increase can be attributed mostly to their compromised immune system and reduced levels of estrogen (Huang *et al*., 2022). UTIs can be caused by many microorganisms, with bacterial UTIs being particularly dangerous and commonly found in both hospitalized and non-hospitalized individuals (Patel *et al*., 2019). Among these bacteria, *Staphylococcus saprophyticus* stands out as a Gram-positive, non-hemolytic and coagulase-negative bacterium. It is commonly responsible for uncomplicated UTIs, particularly in sexually active young women. Less infrequently, it can also contribute to complications consequences such as urethritis, prostatitis, epididymitis and acute pyelonephritis (Nishimura *et al*., 2020).

Notably, *S. saprophyticus* ranks as the second most frequent cause of UTIs following *Escherichia coli*, accounting for approximately 5-20% of cases in women between the ages of 16 and 25 (Zhang *et al*., 2023). The pathogenicity of *S. saprophyticus* is linked to its ability to avoid the immune system and attach to host cells (Silva *et al*., 2020). When bacteria enter the urinary tract, they encounter various host defense mechanisms, including acidic pH, a cleaning flow, fast-changing nutritional circumstances, high osmolality, and high urea concentrations that maintain cleanliness in the upper urinary tract. Therefore, the virulence factor of uropathogens undermines these defense mechanisms (Marlinghaus *et al*., 2016).

The present study investigated the virulence factors identified in UTI isolates of *S. saprophyticus,* specifically focusing on some surface protein genes. Among them, the staphylococcal collagen-binding protein (*sdrI*) binds to the collagen found in the tissues of the host. The uroepithelial adherence factor A (*UafA*) serves as an adhesion facilitating attachment to the cells of the bladder. Hemagglutinin-autolysin adhesin (*Aas*) binds to human ureters and fibronectin. Additionally, the protein surface-associated lipase (*Ssp*) plays a crucial role in causing infection in a murine model and is present in more than 90% of

isolates (Govindarajan and Kandaswamy, 2022; Mancuso *et al*., 2023). Besides, the existence of D-serine deaminase (DsdA) as well as urease is essential for the survival of organisms in the bladder environment (Brauer *et al*., 2019). Currently, there have been no reports implemented to detect the prevalence of virulent factors associated with *S. saprophyticus* in Baghdad. For this purpose, the current investigation aimed to evaluate the prevalence of *S. saprophyticus* and virulence genes linked to UTIs among women in Baghdad, Iraq.

MATERIALS AND METHODS

Sampling and Isolation of *S. Saprophyticus*

Totally, 300 midstream urine samples were taken from women who were hospitalized with suspected UTIs and women who were referred to Karama Teaching Hospital and Al-Elwea Maternity Hospital in Baghdad, Iraq, between the periods of February to August 2023. The women ranged from 18 to 50 years old and had no prior history of genitourinary abnormalities, recent antibiotic use, or underlying illnesses. The midstream method was employed to obtain urine samples from women who had been trained to use the toilet. Afterwards, the samples were immediately transferred to the Biotechnology Research Center of Al-Nahrain University for bacteriological analysis.

Once at the laboratory, the samples were inoculated onto eosin methylene blue agar and blood agar incubated at 37°C for 24 hours. The microbial growth was considered negative if less than 10^3CFU/mL according to the exclusion criteria. A positive, culture was classified as one with monomorphic bacterial growth greater than 10^5 CFU/mL according to the exclusion criteria. Afterward, the suspected colonies were confirmed as *S. saprophyticus* according to standard microbiological characteristics like catalase, coagulase, Gram stain and biochemical identification such as DNase, novobiocin test, trehalose, sucrose maltose, xylose, hemolysin production, urease, nitrate reduction and mannitol fermentation tests (Ferreira *et al*., 2012). Moreover, the Vitek 2 Compact System was used for the final determination.

Extraction of DNA

The genomic DNA extraction was conducted using a DNA extraction kit (QIAGEN, USA), following the manufacturer's recommended protocols for Gram-positive bacteria.

PCR Analysis

All *S. saprophyticus* colonies detected by biochemical examination were verified using *S. saprophyticus*-specific primers, as mentioned previously (Kleine *et al*., 2010). The amplification was conducted in a 20 µL mixture comprising 5 µL of template DNA, 2µL of 10x EasyTaq® Buffer, 1µL of each primer, 1.6µL of a solution containing 2.5 mM of deoxyribonucleotide triphosphates, 0.2µL of EasyTaq® DNA Polymerase, and nuclease-free water which was added up to 20µL. The amplification processes were conducted using a Mastercycler nexus gradient thermocycler (Eppendorf, Germany) with the following protocol: Initial denaturation for 3 minutes at 94°C, then 40 cycles of denaturation for 1 second at 95°C. After that, annealing for seconds at 55°C, extension for 30 seconds at 72°C, and concluding with a final extension at 72°C for 3 minutes.

Multiplex PCR

The clinical isolates of *S. saprophyticus* were subject to Multiplex PCR techniques to identify selected virulence genes, including *Ssp, dsdA UafA, sarA, sdrl, rot, Aas, capD,* and *agr* as described previously by Alo *et al*., (2020) The primer sequences used in the current investigation are provided in Table 1. In brief, the reaction mixtures were conducted in a 20µl, which included the following components: 1.6 µL of dNTPs (2.5 mM), 5 µL of DNA template, 1 µL (5 µM) of both primers, 0.2 µL of *EasyTaq* 10x®DNA Polymerase, 2 µL of 10x *EasyTaq*® Buffer. The final volume was adjusted to 20µl by adding nuclease-free water. The amplification operations were conducted by a Mastercycler nexus gradient thermocycler (Eppendorf, Germany) as follows: The initial denaturation will be performed at 94°C for 5 minutes. The final extension step will be carried out at 72°C for 7 minutes and 35 cycles. Protocol one: (*capD, Aas*): 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds. Protocol two (*Sdrl, dsdA*): 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 min. Protocol three (*Ssp, UafA*): 94°C for 30 seconds, 55°C for 30 seconds, 72°C 2 min. After gel electrophoresis on a 1.5% agarose gel (Merck, Darmstadt, Germany), the products were subjected to 1% ethidium treatment and bands were photographed using the ImageMaster VDS software (Amersham Pharmacia Biotech). A 100 bp DNA ladder was used to compare the sizes of the amplification products

RESULTS

The Prevalence of *S. saprophyticus* in urine Samples

In the present study, the *S. saprophyticus* isoltes were identified and confirmed through a combination of morphological examination, culture characteristics on Blood agar and EMB agar media, and biochemical reaction tests. All isolates showed resistance to novobiocin and were characterized as Gram-positive cocci. Additionally, they tested positive for both catalase and urease. Finally, PCR assays targeting the *S. saprophyticus* species-specific primer were conducted on all putative isolates. The PCR amplification successfully detected 95 (31.66%) strains of *S saprophyticus* out of the 300 urine samples tested. Other isolates were not verified to be strains of *S. saprophyticus*.

Screening of *S. saprophyticus* genes

All isolated strains from urine samples were tested using Multiplex for the prevalence of *Ssp*, *dsdA*, *UafA*, *sarA*, *sdrl, rot*, *Aas*, *capD*, and *agr*. PCR amplification revealed the presence of these genes in the UTIs isolates. The PCR products for the *Ssp*, *Aas*, *sarA*, *capD*, *rot*, *dsdA*, and *agr* genes were 3164, 505, 236, 604, 237, 1299 and 702bp, respectively. A comparison of virulence gene frequencies among the urine isolates showed different distribution patterns of these genes. Of the 95 *S. saprophyticus* isolates, 25 (26.31%) had a single uropathogenic gene. However, the findings revealed that *UafA* gene was detected in 95 (100%) of the isolates (Figure. 1) and 80 (84.2%) harbored the *Ssp* gene (Figure. 2), 90 isolates (94.74%) harbored *Aas* gene (Figure. 3), whereas *dsdA* gene was identified in only 19 (20%) of the isolates (Fig. 3). Regarding regulatory genes, all 95 isolates (100%) harbored *sarA*, *rot*, and *agr* genes, as shown in Figure 4. Conversely, *SdrI* and *CapD* genes were not observed among the isolates examined in the current study.

DISCUSSION

UTIs is a highly prevalent infectious disease worldwide, ranking second in frequency after respiratory disorders (Mlugu *et al*., 2023). According to research, UTIs affect approximately 150 million individuals worldwide each year. Physiological differences and other factors make women more susceptible to UTIs. Approximately half of women and around one-eighth of men develop symptomatic UTIs throughout their lifetimes (Ala-Jaakkola *et al*., 2022). Most UTIs are bladder-related, typically resulting from bacteria ascending the urethra and entering the bladder. If left untreated, the bacteria may reach the kidneys, leading to pyelonephritis, which scares the kidneys and impairs kidney function (Altman and Bagwell, 2018).

Uropathogenic bacteria possess numerous virulent factors that adhere to their cellular surface, facilitating their attachment to tissue receptors and uroepithelial cells. Therefore, treating UTIs must emphasize preventing bacteria from sticking together, which is necessary for the attachment in the first place (Whelan *et al*., 2023). The present results indicated that 95 (31.66%) of the urine samples analyzed were carriers of *S. saprophyticus.* These findings align with previous studies worldwide, which reported 36.1% and 31% in Tunisia and Iran, respectively (Dziri *et al*., 2016; Abedin *et al*., 2021).

S. saprophyticus is associated with urinary tract colonization through the presence of at least six adhesions including surface protein genes *Ssp, Aas*, *Sdrl* and *UafA*. Additionally, enzymes urease and DsdA along with regulatory genes have also been evaluated in this study. The surface-associated lipase *Ssp* was found to be responsible for the formation of a microbial fuzzy surface layer. It has been proposed that lipases serve a function in nourishment or the synthesis of free fatty acids, which might enhance adhesion and so serve a significant part in the persistence and colonization of resident microorganisms on the skin (Wang *et al*., 2021). In the current study, The *Ssp* gene was detected in 84% of *S. saprophyticus* isolates; this is comparable to the findings of Rafiee and Ghaemi, (2023), who identified the occurrence of *Ssp* genes in 80% of urine isolates from women with UTIs. Conversely, Al-Waeely *et al*., (2015) found that all isolates examined in their study possessed this gene. Although the protein's adhesive action in *S. saprophyticus* has not been proved, it is currently observed that Ssp is essential for the survival of living things in a UTI model (Alo *et al*., 2020).

In this study, The *UafA* gene was identified in 100% of *S. saprophyticus* strains in this investigation. This finding is consistent with previous studies that confirmed all isolates include the *UafA* gene (Kline and Lewis, 2017; de Paiva *et al*., 2018). Conversely, Alo *et al*., (2020), demonstrated that *UafA* was absent in all tested isolates. Moreover, the current results revealed that 94% of the isolates harbored *Aas*. The results align with Rafiee and Ghaemi, (2023), who found that 90% of isolated women with UTIs in Iran carried *Aas* gene.

Table 1. Primers are utilized in this work.

Target gene		Sequence primer (5'→3')	Ampliconsize(bp)	References
Aas	F	CAGGTACCGTTAAAGTAC	505	(Kleine *et al.*, 2010)
	R	GATACAACTAACTTGGCAG,		
agr	F	AATGCGAACCAAATATGCC	702	(Kleine *et al.*, 2010)
	R	GTGCAATCAATCGATGCG		
sdrI	F	GGATAAAAATAGCACAATCGACGAA	1624	(Kleine *et al.*, 2010)
	R	CAAGGCTATATTTAGGTGTT		
Ssp	F	AAATTCAGAGAATTAGTAGCC	3164	(Kleine *et al.*, 2010)
	R	ATGAAGAGTTACGTTCACAC		
UafA	F	CGCGGATCCCCAACATCAGAAGTATATGG	2267	(Kleine *et al.*, 2010)
	R	GCGAAGCTTGTGTCAGAAACTA AACCAGC		
dsdA	F	AACGATTTAGCAACACTT	1299	(Kleine *et al.*, 2010)
	R	CTATAAGCAAGA TTTACC		
capD	F	CGTTCAAGATAAAGAGCG	604	(Kleine *et al.*, 2010)
	R	TTCACCAGATCTAATGCC		
sarA	F	CTTATATTAGCGAACACG	236	(Kleine *et al.*, 2010)
	R	GTTAGCTTCTTTAATGCG		
rot	F	TGTTGAAAGATATCGAGG	237	(Kleine *et al.*, 2010)
	R	AATGGATAATAACTGTACG		

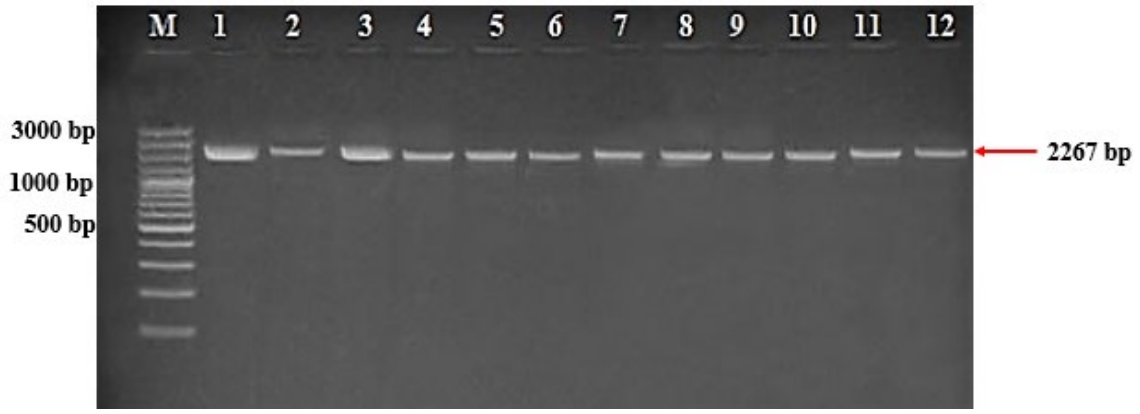

Figure 1. Gene amplification electrophoresis for the *UafA* gene (2267 bp). Visualization was performed using 1.5% agarose electrophoresis at 75 V for 1 hour. Lane M: DNA size marker. Lanes 1–12 represent positive samples for *the UafA* gene**.**

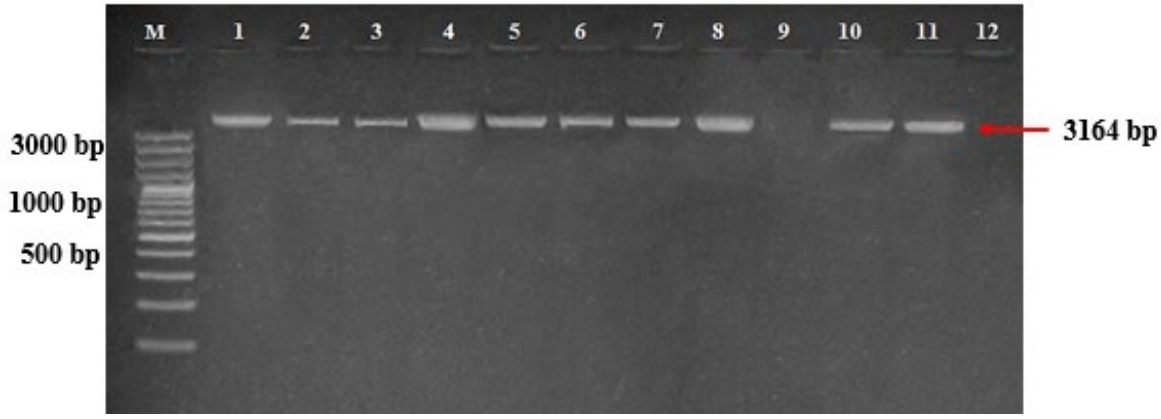

Figure 2. Gene amplification electrophoresis for the *Ssp* gene (3164 bp). Visualization was performed using 1.5% agarose electrophoresis at 75 V for 1 hour. Lane M: DNA size marker. Lanes 1–12 indicate positive samples for the *Ssp* gene.

Figure 3. Gene amplification electrophoresis for *dsdA* gene (1299bp). Visualization was performed using 1.5% agarose electrophoresis at 75 V for 1 hour. Lane M: DNA ladder**.** Lanes 1–12 display positive samples for the *dsdA* gene.

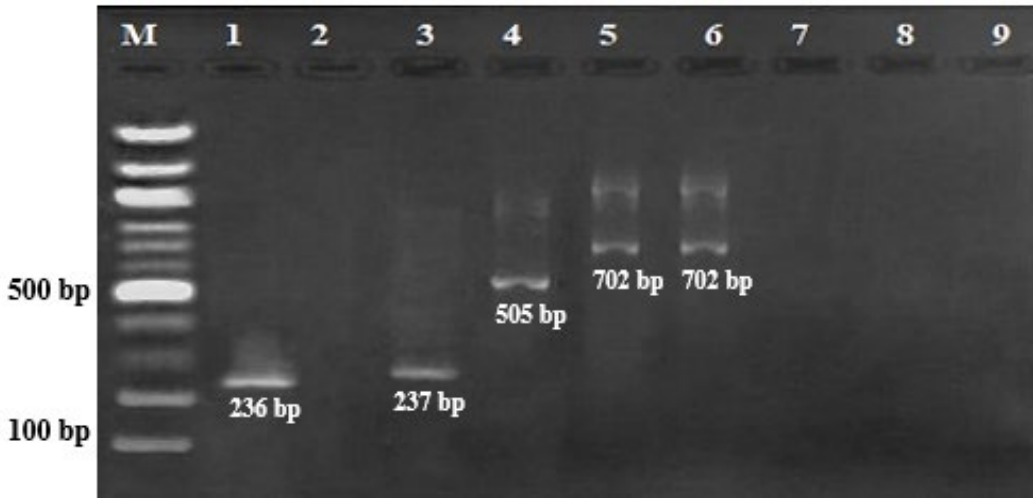

Figure 4. Gene amplification electrophoresis for the *sarA* (236 bp), *rot* (237 bp), *Aas* (505 bp), and *agr* (702 bp) genes. Visualization was performed using 1.5% agarose electrophoresis at 75 V for 1 hour. Lane M: DNA ladder. Lanes1: *sarA* gene. Lane 3: *rot* gene. Lanes 4: *Aas* gene. Lanes: 5 and 6 for *the agr* gene.

The presence of both virulence traits and constitutive function appears to provide an advantage for the bacteria and promote the preservation of the adhesion. Unlike other surface proteins examined, the gene responsible for coding the *SdrI* protein was not detected in our analysis. This observation is consistent with previous findings (Rafiee and Ghaemi, 2023). Further investigations confirmed that *SdrI* binds fibronectin and is essential for the hydrophobicity of microbial surfaces (Kline *et al*., 2015). Therefore, the absence of this gene in infecting strains in this investigation adds to the evidence that its absence does not prevent the bacteria from colonizing the host. Consequentially, Additional investigation is required to ascertain the precise involvement of this gene in the severity and clinical signs of UTIs caused by *S. saprophyticus*.

Human urine has significant amounts of amino acid D-serine and exhibits bacteriostatic or poisonous properties against numerous bacteria. Nevertheless, the existence of this enzyme breaks down D-serine and prevents the organism from experiencing growth impairment in urine (Sasabe and Suzuki, 2018). However, only 20% of the isolates exhibited the presence of *dsdA* in the current study. This contrasts with another investigation conducted in Brazil, which found a 100% occurrence of *dsdA* in all isolates obtained from UTI samples (de Paiva *et al*., 2018).

Nevertheless, all the strains included in this study exhibit urease, which could account for their ability to survive in urine. *CapD* was absent in all isolates examined in the present investigation.

The presence of the regulatory genes *sarA, rot* and *agr* has been observed in *S. saprophyticus*, although their regulatory role in this organism has not been proven. However, the present findings indicated that genes *sarA, rot,* and *agr* were detected in 100% of the examined isolates. This agrees with Kleine *et al*., (2010), who indicated the presence of these genes in all tested isolates. In contrast, a study conducted by Alao *et al*., (2020) in Lagos State, revealed significantly lower occurrence rates for these genes, with 49% for *sarA* gene, 6% for *rot* gene and 3% for the *agr* gene. These discrepancies underscore potential regional variations in the prevalence of these uropathogenic factors in isolates.

The pathophysiological cause of *S. saprophyticus* infection is multifactorial, which requires many virulent genes involved in the uropathogenic existence of UT. Therefore, a comprehensive review of the uropathogenic virulence genes involved in UTIs is required. It is also important to highlight the importance of routine surveillance of UTI-causing pathogens, including Staphylococcus spp., to monitor prevalence and resistance among patients with similar circumstances as those reviewed in this study.

CONCLUSION

The current data successfully demonstrated a high incidence of uropathogenic virulence genes among *S. saprophyticus* isolates in the urine samples examined. To our knowledge, the first study, which examined *S. saprophyticus* strains, associated with UTIs for a potential uropathogenic nature of those virulent genes that have proven to be important in other gram-positive and via-negative bacteria causing these infections. If these bacteria enter the urinary tract system, then it will be a very big problem to dispose of them and get rid of their presence. Consequently, it is important to apply proper health preventive measures to prevent the further spread of this bacterium among organisms such as plasmids, which can confer resistance to antibiotics and are a serious public concern.

DECLARATION

I hereby confirm that the manuscript has all my own tables and figures, and there are no conflicts of interest.

ETHICAL APPROVAL

The local ethical commission at Al-Nahrain University, Iraq, approved the undertaking (Approval No.096412951).

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