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ASSESSMENT OF TRIMETHOPRIM -LOAD SOLD LIPID NANOPARTICLES ACTIVITY AGAINST STAPH AUROUS ISOLATED FROM URINARY TRACT INFECTIONS IN WOMEN

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Abstract

Objective: to Isolate Staph aurous from urinary tract infection in women and study in the vitro antibiotic of the standard Trimethoprim and Trimethoprim loaded solid lipid nanoparticles formulation against Staph aurous isolated.

Method: A total of 123 urine samples from women patients with urinary tract infections were gathered, from different hospitals around waist province from 15 June for 15 September, 2023. Morphological characteristics and biochemical tests based on Api20, Vitek 2 system, and Bogy's Manual of Systematic Bacteriology were used to identify the samples

Result: The results were 60 (48.7%) isolates of Staph aurous obtained from 123 from women with urinary tract infections (UTI), distributed as symptomatic 71(57.7%) and asymptomatic 52 (42.3%). In-vitro Antibacterial Activity the activity of TMP-SLNs formulation and standard TMP against UTI isolates of Staph aurous was sensitive to the tested formulations. The TMP-SLNs formulation shown considerably greater ($p<0.05$) antibacterial activity in terms of zone of inhibition against the bacteria. When compared to typical TMP values, the MIC and MBC values of the TMP-SLNs formulation against Staph. aurous were almost sixteen times higher. This indicates that TMP's antibacterial action is enhanced when it is loaded into SLNs.

Conclusions: the result of study highlight that Trimethoprim -load sold lipid nanoparticles it might be an excellent option for the sustained delivery of Antibacterial against Staph aurous isolated.

Keywords: sold lipid nanoparticles, Staph aurous, Trimethoprim.

Introduction

Women of childbearing age are most likely to experience acute, uncomplicated cystitis as a result of urinary tract infections (UTIs), which are frequently caused by bacteria (1). Even though acute, simple cystitis is not considered a dangerous ailment, the estimated six days of discomfort it causes the patient negatively impacts their quality of life (2). Gram-positive Staph. aurous is a non-spore-forming, grape-forming, non-motile pathogen that forms clusters. It is mostly caused by coagulase, a Staphylococci positive pathogen that is characterized by invasiveness, toxin mediated virulence, and antibiotic resistance (3). Some S. aurous strains are

resistant to drugs (4) As colloidal carrier systems, solid lipid nanoparticles (SLNs) provide the benefits of conventional systems without some of their main drawbacks (5). Among the benefits of SLNs are the ability to incorporate hydrophilic and lipophilic drugs, the ability to target and control drug release, the ability to increase drug stability, a high drug payload, the lack of bio toxicity in the carrier, the ability to sterilize, and good tolerability (6). The Objective of study focuses on the isolation and characterizations of *Staph.aurous* isolated from UTIs in women and study in the vitro and vivo antibiotic of the standard Trimethoprim and Trimethoprim loaded solid lipid nanoparticles formulation against Staph.aurous isolated.

Materials and Methods

123 of urine samples were collocated from women, from various waist-province hospitals (Al-Kut hospitals for Gynecology, Obstetrics, and Pediatrics) between June 15 and September 15, 2023.Morphological traits and biochemical tests based on Api20O, the Vitek 2 method, and Bogy's Manual of Systematic Bacteriology were used to identify the isolator.

Bacterial spp. of Study:

Pathogenic bacterial isolators Of *Staph. Aurous* wore used from the simple's that collocated from women afflicted with UTI symptoms from various hospitals in the Wasit Province (Al-Kut Hospital for Gynecology and Obstetrics and Pediatrics).

Making the Standard Bacterial Suspension:

Using the Standard McFarland solution No.0.5, the average number of live Staph aurous organisms per milliliter of the stock suspensions was calculated. After removing 1 milliliter from the bacterial suspension culture (nutrient agar) and washing it with 9 milliliters of peptone water, then serially diluting 1 milliliter of this suspension ten times. Following these steps, standard McFarland solution No. 0.5 was made: 100 milliliters of distilled water were used to dissolve 1.175 grams of barium chloride (BaCl2.2H2O) to create solution (A). One milliliter of concentrated sulfuric acid (H2SO4) was added to one hundred milliliters of distilled water to create solution (B). 0.5 ml of solution A was added to 99.5 ml of solution B to combine the two solutions. An approximate cell density of 1.5 $\times 10^8$ cell/ml was obtained by comparing the turbidity of two bacterial suspensions using the produced solution.

In-vitro antibacterial study of Trimethoprim and TMP-SLNs:

In accordance with (7), the agar well diffusion method was used to evaluate the evaluated forms' antibacterial activity. For every 20 milliliters of sterile nutritional agar, 0.2 milliliters of standardized bacterial stock suspensions (1.5 $\times 10^8$) cell/ml of Staph aurous were carefully mixed. 20 milliliters of the nutrient agar that had been inoculated were added to sterile petri plates. A sterile cork borer was used to create four 8 mm diameter wells in each plate after the agar had had time to set. The wells were then filled with 0.1 ml of each concentration of 1, 2, 4, 8, 16, 32, 64, 125, 250, 500, and 1000µg/ml of TMP for both, regular TMP and TMP-SLNs using a microliter pipette and allowing them to diffuse for two hours at room temperature. After that, the plates were incubated for 24 hours at 37 Ċ while upright. For every concentration, three replicates were used, and the diameter of the inhibition zone surrounding each well was

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measured in millimeters in relation to each tested organism to assess the activity of the tested form agents. The composition of addition blank SLNs and D.W. were utilized concurrently as positive and negative controls, respectively. The outcomes were summarized together with the standard errors mean values

Determination of MIC and MBC:

The broth dilution method was used to calculate the MIC and MBC of TMP-SLNs and standard TMP against E. coli. Following the preparation of TMP stock solutions at 1000 µg/ml for various TMP-SLNs formulations and standard TMP, the solutions were further diluted in 4 mL of Muller-Hinton broth to provide concentrations ranging from 1 to 1000 µg/mL. After adding 50 µL of bacterial inoculums, the final concentration of bacteria in each tube was adjusted to approximately 5×10^6 CFU/mL**.**additionally employed as controls were the standard saline solution and the blank SLNs formulation. The lowest medication concentration that could prevent observable bacterial growth was found by looking for any signs of turbidity in the test tubes following a 24-hour incubation period at 37 °C. The MBC was then determined by subculturing the bacteria from the MIC broth tubes onto new agar plates. The lowest medication concentration that prevented the examined bacteria from growing was the in vitro MBC value (8).

Statistical Analysis:

the details of current study was statistically evaluated using the two-way ANOVA, least significant differences (LSD), and chi-square tots (X2) in the Statist Package for Social Science (SPSS) version 27 program. The significance threshold was set at five percent. P0.05 was seen as noteworthy at midday.

Result and Dissociation

Prevalence of Staph aurous Based on Collected Simples:

The results of the study appeared in table (1). Figure (1), 60 (48.7%) isolates of *Staph. aurous* were obtained from 123 from women with urinary tract infections (UTI), distributed as symptomatic 71(57.7%)and asymptomatic 52 (42.3%).The prevalence of *Staph. aurous* was among symptomatic urinary tract infections 39 (65%) and in asymptomatic urinary tract infections was 21 (35%) .

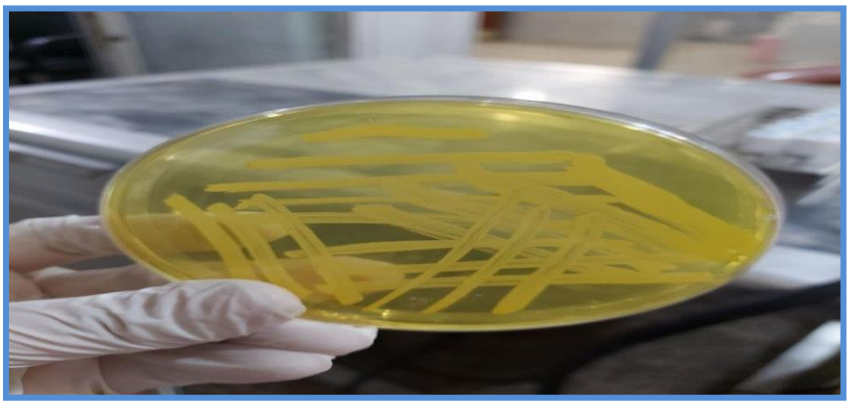

Fig. (1). *S.aureus* growth on mannitol salt agar.

Table (1):- Distribution of *Staph. aurous* isolates according to collected samples.

Age group	Symptomatic Simples	*Staph. aurous* isolates	Asymptomatic Simples	*Staph. aurous* isolates	Total isolates
15-19	10	5	11	2	7
20-24	16	8	15	9	17
25-29	27	16	15	6	22
30-34	10	6	5	2	8
35-39	6	4	4	1	5
40-45	2	0	2	1	1
Total	71(57.3%)	39(65%)	52(42.7%)	21(35%)	60 (48.7%)
X^2		3.48		17.03	
P valve		0.626		0.004*	

* Significantly difference at P<0.05

Table 1 presents the results, which indicate that women with symptomatic UTIs had a significantly higher prevalence of Staph. aurous than those without symptoms. The highest number of isolates (20) was found in the symptomatic age group of 25–29 years, while the highest number of isolates (13) was found in the asymptomatic age group of 20–24 years. According to a study conducted in Egypt by (9), women between the ages of 21 and 30 had the highest frequency of Staph. aurous. This higher presence in this age group may be related to women's increased sexual activity, which can cause minor urethral damage and transfer bacteria from the perineum to the urethra and bladder, making them more vulnerable to UTIs (10). However, Staph. aurous was discovered in 10% of the samples by Odongo *et al.* (11) in Uganda, and 52% of these samples contained Staph. aurous isolates from women as opposed to 48% from men. The causes for the variations in Staph. aurous prevalence could be attributed to various factors such as geography, health concerns, social customs, economic status of the community, cleanliness and awareness, time and quantity of sample collection, and use of antibiotics before to simple collection (12). Staph. aurous the near closeness of the anus to the urethral tube may account for the increased presence in women compared to men. Additionally, women's urethral tubes are shorter than men's, which reduces the distance germs must travel to reach the bladder (13). The existence of Staph aurous may have helped the microbe adapt to the hostile environment of the urinary tract. The bacteria's ability to spread from the anus, which is its natural habitat, to the urethral tube due to their close proximity to one another may be one of the most important virulence factors, as it aids in the bacteria's ability to not only survive but also cause infection and disease(14).

Antimicrobic Action in vitro:

The Antimicrobic Action of TMP-SLNs formulation and standard TMP against UTI isolates of *Staph. aurous* are shown in table 2 .It was found *Staph.aurous was* sensitive to the tested formulations. the TMP-SLNs formulation hown considerably greater ($p<0.05$) antibacterial activity in terms of zone of inhibition against the tested microorganisms, the first result of inhibition was achieved at 4µg/ml, while the first zone of inhibition for standard TMP was recorded at 64µg/ml. At 1000 µg/ml TMP-SLNs given higher antibacterial activity in

comparing with standard TMP (30 and 20mm respectively).In general, both formulations showed antibacterial activity with increasing their concentration of TMP, Following this, the growth of the bacteria was suppressed, indicating that the antibacterial action is associated with the drug's encapsulation. The formulation that was evaluated, SLNs, did not exhibit any zone of inhibition against the tested bacteria when employed as a positive control.

Table 2:- The Antimicrobic Action TMP-SLNs formulation and standard TMP against UTI isolates of *Staph. aurous* bacteria expressed as zone of inhibition (mm).

Concentration µg/ml	**TMP-SLNs (Inhibition zone-mm)**	**TMP (Inhibition zone-mm)**
	Mean ±SE	**Mean ±SE**
1	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00
4	12.00 ±0.57	0.00±0.00
8	14.33 ±0.33	0.00±0.00
16	16.00±0.57	0.00±0.00
32	18.33±0.33	0.00±0.00
64	20.66±0.37	10.00±0.00
125	22.33±0.66	12.00±0.00
250	24.37±0.43	15.00±0.57
500	26.75±0.40	16.00±0.66
1000	30.23±0.45	21.00±0.33
+ve control (SLNs)	0.00±0.00	0.00±0.00
-ve control (DW)	0.00±0.00	0.00±0.00
LSD(P<0.05)	0.54	

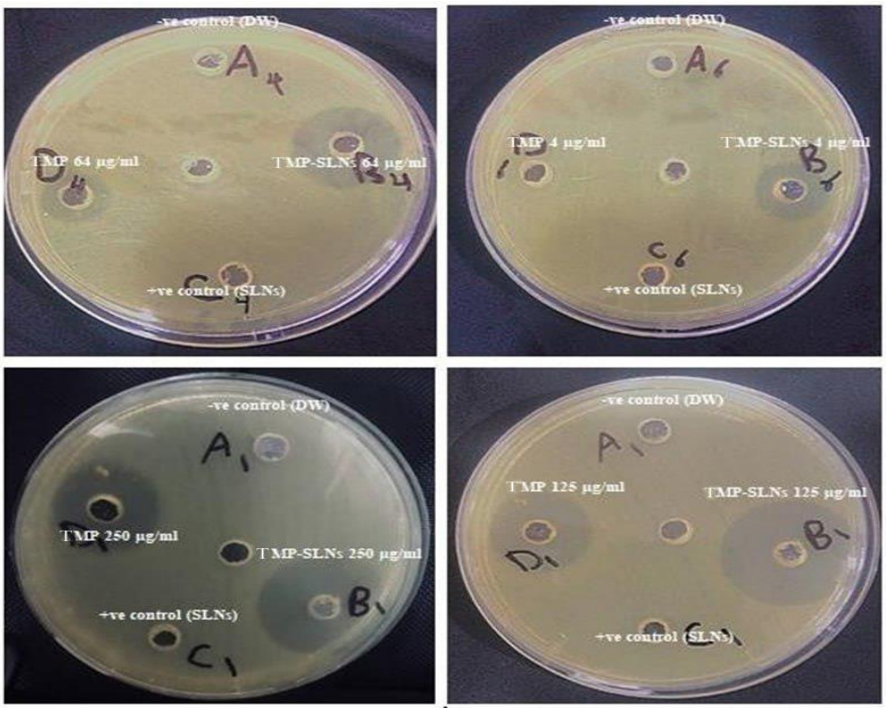

Figure (2)-: In-vitro antibacterial activities of different concentrations of TMP-SLN, standard TMP, +ve control, and -ve control DW), respectively against *Staph. aurous* bacteria

These findings suggested that TMP-SLNs, as opposed to free TMP, could stop bacterial growth for a longer amount of time. This suggested that, in comparison to free TMP, TMP-SLNs have higher antibacterial activity. These findings may be explained by the tested formulation's lipophilicity, which enhances TMP's cellular entry into the bacterial membrane, as well as the particles' tiny size (14).

Due to their sustained release and greater capacity to penetrate the bacterial cell wall because of their small molecular weight and hydrophobic structure, which is similar to that of gram-negative bacteria, it has been observed that employing nanoparticles to entrap antibacterial agents may improve their activity. They might also successfully lessen the P-gp efflux pumps' activity. Since there was no antibacterial activity in the blank control formulations, the increased antibacterial efficacy can be attributed to these formulations' effective delivery of TMP molecules to the site of (15).observed enhanced antibacterial activities of drug-loaded SLNs (16).

Measuring of MIC and MBC of TMP-SLNs and standard TMP against Bacterial Growths:

The TMP-SLNs formulation's MIC and MBC values (Table 3) against Staph. aurous were almost sixteen times higher than the typical TMP values. This indicates that TMP's antibacterial action is enhanced when it is loaded into SLNs.

Table (3)-: The MIC and MBC result (µg/ml) of TMP-SLNs formulation and standard TMP against UTI isolates of *Staph. aurous* bacteria.

Bacteria		TMP-SLNs	Standard TMP	+ve control (SLNs)	-ve control (DW)
Staph. aurous	MIC	4	64	0	0
	MBC	2	32	0	0

Regarding TMP-SLNs, where they reported the same MIC and MBC values as that of the normal TMP, our results are consistent with (17). Their findings demonstrated that, when compared to TMP (18), the smaller MD values resulted in lower MIC and MBC values of TMP-SLN suspension. The reduced antimicrobial efficaciousness against Staph. aurous The data presented showed the potential advantages of employing nanotechnology to lower antibiotic dosages and lower the likelihood of bacterial strains developing antibiotic resistance.

Conclusions:

The result of study highlights that Trimethoprim -load sold lipid nanoparticles could be have a strong chance of providing delivery of Antibacterial against *Staph.aurous* isolated from urinary tract infection

References

1. Zagaglia, C., Ammendolia, M. G., Maurizi, L., Nicoletti, M., & Longhi, C. (2022). Urinary Tract Infections Caused by Uropathogenic *Staph. Aurous* Strains—New Strategies for an Old Pathogen. Microorganisms, 10(7), 1425.

2. Shawki, A., & McCole, D. F. (2017). Mechanisms of intestinal epithelial barrier dysfunction by adherent-invasive *Staph.aurous*. Cellular and molecular gastroenterology and hepatology, 3(1), 41-50.

3.Singha, P. and Prakash, A. (2008). Isolation of Staphylococcus aureus and Listeria monocytogenes from milk products sold under market conditionat Agra region. ActaagriculturaeSlovenica. 92(1): 83-88.

4.Tao, Y., Yang, F., Meng, K., Chen, D., Yang, Y., Zhou, K., ... & Xie, S. (2020). Exploitation of enrofloxacin-loaded docosanoic acid solid lipid nanoparticle suspension as oral and intramuscular sustained release formulations for pig. Drug Delivery, 26(1), 273-280.

5.Thatipamula, R. P., Palem, C. R., Gannu, R., Mudragada, S., & Yamsani, M. R. (2019). Formulation and in vitro characterization of domperidone loaded solid lipid nanoparticles and nanostructured lipid carriers. Daru: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences, 19(1), 23.

6. Sivakumar, S.(2022). Therapeutic potential of chitosan nanoparticles as antibiotic delivery system: Challenges to treat multiple drug resistance. Asian J. Pharm. Free Full Text Artic. Asian J. Pharm, 10, 2.

7.Rehman, M.; Ihsan, A.; Madni, A.; Bajwa, S.Z.; Shi, D.; Webster, T.J.; Khan, W.S. (2020). Solid lipid nanoparticles for thermoresponsive targeting: Evidence from spectrophotometry, electrochemical, and cytotoxicity studies. Int. J. Nanomed., 12, 8325.

8. Mukherjee, S., Ray, S., & Thakur, R. S. (2019). Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian journal of pharmaceutical sciences, 71(4), 349.

9.Raeispour, M., & Ranjbar, R. (2018). Antibiotic resistance, virulence factors and genotyping of Uropathogenic Escherichia coli strains. Antimicrobial Resistance & Infection Control, 7(1), 1-9.

10. Rajivgandhi, G., Vijayarani, J., Kannan, M., Murugan, A., Vijayan, R., & Manoharan, N. (2014). Isolation and identification of biofilm forming uropathogens from urinary tract infection and its antimicrobial susceptibility pattern. Int. J. Adv. Lif. Sci, 7(2), 352-363.

11. Derese, B., Kedir, H., Teklemariam, Z., Weldegebreal, F. Balakrishnan, S. (2016). Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending at antenatal Clinic in DilChora Dire Dawa, Eastern Ethiopia. Therapeutics and Clinical Risk Management, 12: 251 260. .DOI: 10.2147/TCRM.S9983.

12 Normanno, G., Firinu, A., Virgilio, S., Mula, G.,Dambrosio, A.,Poggiu, A., Bolzoni, G. (2005). Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *International Journal of Food Microbiology*, *98*(1):73–79.

13. Saleem, A. J., Nasser, N. E., and Ali, M. R. (2016). Prevalence of genes encoding enterotoxins and exfollarive toxins among methicillin-resistant *Staphylococcus aureus* clinical isolates in Iraq. *World Journal of Pharmaceutical Research*, *5*(7):208–216.

14.Sande, L., Sanchez, M., Montes, J., Wolf, A. J., Morgan, M. A., Omri, A., et al. (2019). Liposomal encapsulation of vancomycin improves killing of methicillinresistant Staphylococcus aureus in a murine infection model. J. Antimicrob. Chemother. 67, 2191–2194. doi: 10.1093/jac/dks212.

15.Alihosseini, F., Ghaffari, S., Dabirsiaghi, A. R., & Haghighat, S. (2015). Freeze drying of ampicillin solid lipid nanoparticles using mannitol as cryoprotectant. Brazilian journal of pharmaceutical sciences, 51, 797-802.

16.Bayón-Cordero, L., Alkorta, I., & Arana, L. (2019). Application of solid lipid nanoparticles to improve the efficiency of anticancer drugs. Nanomaterials, 9(3), 47

17.Chetoni, P., Burgalassi, S., Monti, D., Tampucci, S., Tullio, V., Cuffini, A. M., ... & Cavalli, R. (2016). Solid lipid nanoparticles as promising tool for intraocular tobramycin delivery: Pharmacokinetic studies on rabbits. European Journal of Pharmaceutics and Biopharmaceutics, 109, 214-223.

18.Al-Qushawi, A., Rassouli, A., Atyabi, F., Peighambari, S. M., Esfandyari Manesh, M., Shams, G. R., & Yazdani, A. (2016). Preparation and characterization of three tilmicosin-loaded lipid nanoparticles: physicochemical properties and in-vitro antibacterial activities. Iranian Journal of Pharmaceutical Research: IJPR, 15(4), 663.

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