Global Scientific Journal of Biology 9 (1) 2024/ 128-133

Contents lists available at www.gsjpublications.com

Global Scientific Journal of Biology
Part A: Microbiology and Biotechnology
ISSN: 2524-227X
journal homepage: www.gsjpublications.com/gsjb

Study of Antibiotic Resistance in Staphylococcus Lentus Bacteria Isolated from Palm Skin Using the Vitek2 System Version

Ammar Issa Taresh[1*], Ali. F. Hussein[2], Alia Mohammed Abdulatef[1], Rakeshkumar R. Panchal[2], Nada J. Dawood[2], Dweipayan Goswami[2], Kiransinh Rajput[2], Meenu Saraf[2]

[1]Southern Technical University, Technical institute of Basra, Department of Community Health Technical, Iraq.*
[2]Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

ARTICLE INFO

Received: 5 May 2024,
Revised: 13 May 2024,
Accepted: 15 May 2024,
Online: 21 June 2024

Keywords:
Staphylococcus lentus, antibiotic resistance, Vitek 2 system , microorganism isolation, skin, Basra City

ABSTRACT

Staphylococcus lentus (S. lentus) bacteria are a significant concern in causing infectious diseases, known for their rapid transmission among humans and antibiotic resistance. To investigate this, we conducted a study where we collected 100 samples from the palm skin of students at a nursing institute in Basra City between March 1, 2022, and December 1, 2023. From these samples, we isolated microorganisms and identified 15 pure isolates, categorizing them into five distinct types based on their physical characteristics and using the Vitek 2 system. Staphylococcus lentus stood out among these isolates, with a 97% likelihood of presence. Our primary aim is to delve into the antibiotic resistance of S. lentus using the Vitek 2 system, with detailed findings presented in Table 2 and Figure 1b.

1. Introduction

The development of resistance in bacteria poses a significant challenge to public health, as certain bacterial strains can have severe consequences for human well-being. Repeated exposure to therapeutic agents prompts bacteria to adapt by altering their genetic makeup and producing specific chemicals. This phenomenon, known as antimicrobial resistance (AMR), remains a pressing issue in global health, with research on new antibiotics lagging. Bacterial biofilms in various environments, such as hospitals, restaurants, bathrooms, and water treatment plants, pose significant risks [1].

The rise of multidrug resistance in bacterial pathogens presents a unique hurdle for antibiotic treatment. Gram-negative species, in particular, increasingly exhibit resistance mechanisms, often possessing multiple mechanisms that make many drug classes ineffective. Novel approaches are urgently needed to address this challenge. Marc Sprenger, Director of the WHO's Secretariat for Antimicrobial Resistance, recently warned of infections rapidly becoming resistant to life-saving drugs [2], suggesting that we may be on the verge of entering a post-antibiotic era. This alarming trend raises concerns that routine medical

Corresponding author:
E-mail addresses: ammar.issa@stu.edu.iq (Ammar)*, rrpanchal@gujaratuniversity.ac.in (Rakeshkumar), alia.abdullateef@stu.edu.iq (Alia), n.alkamil@stu.edu.iq (Nada), dweipayan.goswami@gmail.com (Dweipayan), drkiransinhrajput@gujaratuniversity.ac.in (Kiransinh), a.faisal@stu.edu.iq (Ali), msaraf@gujaratuniversity.ac.in (Meenu)
doi: 10.5281/gsjb.2024.11636017

procedures and treatments, such as simple surgeries or cancer therapies, could become untenable due to these resistant organisms [3].

Staphylococcus lentus (*S. lentus*) is part of the S. sciuri group, which are Gram-positive bacteria that can thrive with or without oxygen, are coagulase-negative, oxidase-positive, and typically appear as non-motile, non-sporforming cocci either singly, in pairs, or tetrads [5]. S. lentus can be found in various environments, such as soil, sand, water, and hospital settings [6]. Numerous studies have linked S. lentus to various human diseases. For example, it has been isolated from splenic samples of patients suffering from sepsis [7]. Our research on skin infections echoes the findings by Rivera et al. [25], who also discovered (iv) S. lentus in patients with conditions like renal failure and urinary tract infections (UTIs), highlighting its common occurrence in UTI cases [8, 9]. To confirm the identity of isolates, we utilized the automated VITEK 2 compact system with GP-ID cards, providing precise and accurate identification at both the genus and species levels.

The current study was structured with the following specific goals:

(i) To isolate and describe Gram-positive bacteria obtained from skin balm.

(ii) (To pinpoint the presence of *Staphylococcus lentus*.

(iii) To investigate the antimicrobial resistance patterns exhibited by pathogenic bacteria, particularly focusing on *Staphylococcus lentus*.

2. Materials and Methods

MEDIA USED

All media and glassware were sterilized using an autoclave at a pressure of 15 lb. inch^{-2} and a temperature of 120°C for 20 minutes.

Medium/Reagent	Composition
Soft Agar	Agar (0.5-0.8%)
Nutrient Broth (pH 7.0)	Peptone (10 g/L), Beef extract (3 g/L), Sodium chloride (5 g/L), Distilled water (to make 1 L), pH adjusted to 7.0
Nutrient Broth (pH 7.4)	Peptone (10 g/L), Beef extract (3 g/L), Sodium chloride (5 g/L), Distilled water (to make 1 L), pH adjusted to 7.4
Nutrient Agar	Peptone (5 g/L), Beef extract (1.5 g/L), Agar (15 g/L), Distilled water (to make 1 L)
Mueller Hinton Agar	Beef extract (2 g/L), Acid hydrolysate of casein (17.5 g/L), Starch (1.5 g/L), Agar (17 g/L), Distilled water (to make 1 L)
MacConkey Broth	Peptone (17 g/L), Lactose (10 g/L), Bile salts (1.5 g/L), Sodium chloride (5 g/L), Neutral red (0.03 g/L), Crystal violet (0.001 g/L), Agar (15 g/L), Distilled water (to make 1 L)
Blood Agar	Trypticase Soy Agar (TSA) base (40 g/L), Defibrinated sheep blood (5-10%), Distilled water (to make 1 L)
Reagents and Buffers	- Gram staining solution: Crystal violet (0.3%), Iodine (1%), Ethanol or acetone (decolorizer), Safranin or fuchsine (0.3-1%), Distilled water
	- KOH: Potassium hydroxide (3-10%)

VITEK 2 systems version:9.03.4

Card Type: GP, Barcode: 24226166403056575, Testing Instrument: 00001C29FD05(24566)
Card Type: AST-P592, Barcode: 3722504403186269, Testing Instrument: 00001C29FD05(24566)
Card Type: GP, Barcode: 2422616403056578, Testing Instrument: 00001C29FD05(24566)
Card Type: AST-P592, Barcode: 3722504403186267, Testing Instrument: 00001C29FD05(24566)
Card Type: GP-P592, Barcode: 2422616403056577, Testing Instrument: 00001C29FD05(24566)
Card Type: AST-P592, Barcode: 3722504403186268, Testing Instrument: 00001C29FD05(24566)

3. Methods

Collection of Isolates

100 bacterial samples (BA=100) were collected from the palm skin using sterile cotton swabs and promptly transported to the laboratory for initial processing. The time elapsed between sample collection and processing was at most 3 hours. From these samples, fifteen bacterial isolates were obtained in a pure form. Gram stain was utilized to examine the isolated bacteria, enabling the study of their morphology, including gram reaction and shape. Biochemical tests were carried out following the protocol outlined in [18]. Gram-positive (GR+) bacteria were specifically chosen and identified using the VITEK 2 system version.

Bacterial Identification

Staphylococcus lentus isolates were identified using the VITEK 2 system, version [insert version number]. The details of the cards used for testing are as follows:

1. Card Type: GP, Barcode: 24226166403056575, Testing Instrument: 00001C29FD05

2. Card Type: AST-P592, Barcode: 3722504403186269, Testing Instrument: 00001C29FD05 (24566)

These cards were utilized to analyze and identify the *Staphylococcus lentus* isolates within the sample population.

4. Results And Discussion

In Basra city, southern Iraq, *Staphylococcus lentus* bacteria have been identified. From 100 samples collected, 60 were obtained from the palm skin of females and 40 from males. Fifteen distinct bacteria were isolated in pure form. Among these isolates, ten tested positive and five tested negative through Gram staining. Following the procedures outlined in [18], biochemical tests were conducted. Upon morphological and VITEK 2 system analysis, five different types of bacteria were identified from the 15 pure isolates. Notably, *Staphylococcus lentus*, with a 97% probability, was among the identified organisms, characterized by its bionumber 570001403663731. Additionally, Kocuria rhizophila (with a 92% probability) and Kocuria rosea (with a 97% probability) were also identified, each with their respective bionumbers: 000014102000031 and 040011300000000 [12]. *Staphylococcus lentus* is typically regarded as an uncommon contributor to human infections [10,11]. The primary objective of our study was to isolate *Staphylococcus lentus* for further investigation into its antibiotic resistance using the VITEK 2 system. Upon morphological analysis, the isolated colonies exhibited smooth, convex, mucoid characteristics, appearing either non-pigmented or yellow, ranging from pale beige to pale reddish yellow [12]. Regarding growth characteristics, the bacteria displayed slow growth in 10% NaCl and poor growth in 15% NaCl solutions. Colonies were observed to be round, smooth, convex, mucoid, and varied in color, ranging from glistening white to cream or pale beige [12].

The antibiotic sensitivity test on S. lentus revealed resistance to most antibiotics, with high-level (synergy) antibiotics showing resistance against Gentamicin and Streptomycin [12].

The study's findings highlight Staphylococci as the primary culprits behind hemodialysis infections, a trend consistent with previous research [13]. It's noted that infections associated with hemodialysis often stem from coagulase-negative staphylococci. Gram-positive cocci are frequently implicated in bloodstream infections (BSIs) among hemodialysis patients, with *Staphylococcus lentus* identified as one of the causative agents of nosocomial bacteremia [14]. Additionally, S. lentus has been implicated in outbreaks of bloodstream infections in neonatal intensive care units (NICUs) [20]. Coagulase-negative bacteremia is commonly linked to prolonged use of indwelling central venous catheters in hemodialysis patients [16].

Figure 1. Bacterial colonies on nutrient agar (a) and blood agar (b) plates

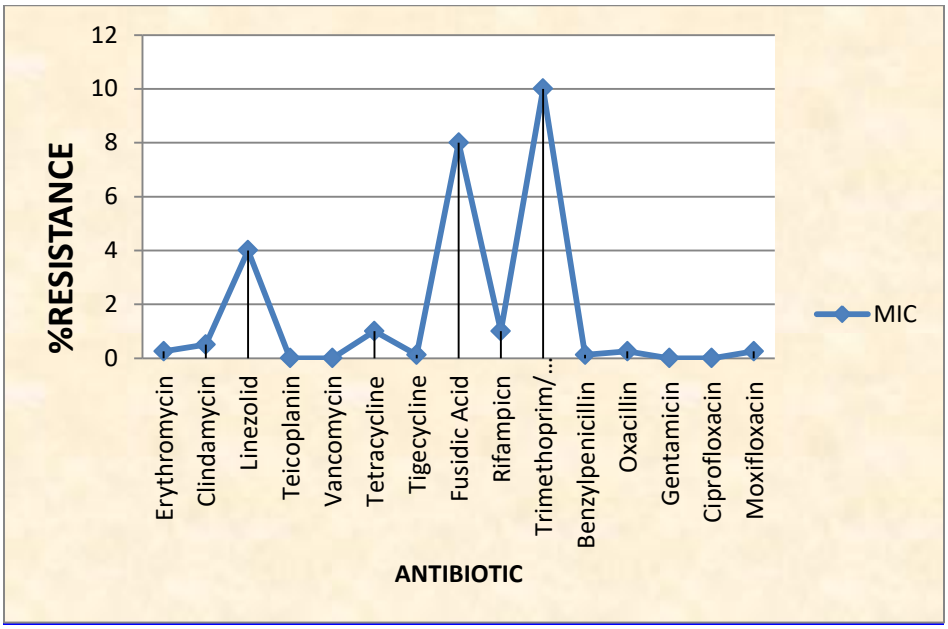

Figure 2. Percentage resistance of bacterial isolates against antibiotics from skin palm students.

Table 1. Skin palm samples used in the study for *Staphylococcus lentus.*

Source	Department of Nursing Technologies	Male Samples	Female Samples
		40	**60**
Total		**100**	

Table 3. Results of Antimicrobial Sensitivity Testing for *Staphylococcus lentus* Using the Automated Microbiological System VITEK 2, Version.

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	**P0S**	**+**	Erythromycin	**<=0.25**	**S**
Benzylpenicillin	**0.12**	***R**	Clindamycin	**0.5**	**S**
Ampicillin			Linezolid	**4**	**S**
Oxacillin	**<=0.25**	***R**	Teicoplanin	**<=0. 5**	**S**
Imipenen			Vancomycin	**<=0. 5**	**S**
Gentamicin high level (synergy)			Tetracycline	**<=1**	**S**
Streptomycin high level (synergy)			Tigecycline	**<=0.12**	**S**
Gentamicin	**<=0. 5**	**S**	Fosfomycin		
Ciprofloxacin	**<=0. 5**	**S**	Fusidic Acid	**8**	**R**
Moxifloxacin	**<=0.25**	**S**	Rifampicn	**1**	**S**
Inducible Clindamycin resistance	**NEG**	**-**	Trimethoprim/ Sulfamethoxazole	**<=10**	**S**

* = AES modified, ** = User modified. MIC Interpretation Guideline: S = sensitive, R = resistance, I = intermediate.

5. Conclusion

This study thoroughly examines the prevalence and antibiotic resistance patterns of staphylococci across diverse skin samples in Basra, southern Iraq. The results emphasize the need for additional experiments aimed at pathogenic bacteria to gain deeper insights into the factors driving their heightened antibiotic resistance. Furthermore, there's a critical need to assess the contribution of biofilms to the development of chronic infections associated with the bacteria under investigation. Such inquiries are essential for devising efficient strategies to address antibiotic resistance and enhance patient care outcomes.

6. References

[1]. Chmagh, Aalaa A., et al. "Anti-Biofilm Activity of Nigella Sativa Extracts Against Pathogenic Bacteria." *HIV Nursing* 23.2 (2023): 804-809.

[2]. Kretschmer, Matthias, et al. "Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus Botrytis cinerea." *PLoS pathogens* 5.12 (2009): e1000696.

[3]. Tillotson, Glenn S. "Trojan horse antibiotics–a novel way to circumvent Gram-negative bacterial resistance?." *Infectious Diseases: Research and Treatment* 9 (2016): IDRT-S31567.

[4]. Laxminarayan R., Chaudhury R.R. Antibiotic resistance in India: drivers and opportunities for action. *PLoS Med.* 2016; 13(3): e1001974.

[5]. Stepanovic, S.; Dakic,I.; Morrision,D.; Hauschild,T. and Devriese. A comparative evaluation of phenotypic and molecular methods in the identification of members of the *Staphylococcus sciuri* group. Syst. Appl. Microbiol, 2005a; 28: 353–357.

[6]. Dakic, I.; D. Morrison, D.; Vukovic, B. Savic; A. Shittu, P.; Jezek,T. Hauschild and S. Stepanovic. Isolation and molecular characterization of Staphylococcus sciuriin the hospital environment. J. Clin. Microbiol, 2005; 43: 2782–2785.

[7]. Karachalios, G.N. ; Di Giannatale, E. ; Salinetti , AP. ; Salandra, G. and Bartoli , M. Splenic abscess due to Staphylococcus lentus:a rare entity. Scand. J. Infect. Dis., 2006; 38: 708–710 .

[8]. Guirguitzova, B., D.; Chankova, and B. Zozikov. Staphylococci asuropathogens— frequency of isolation in hospitalized patients and sensitivityto antimicrobial agents. Ann. Urol. (Paris), 2002; 36: 341–347.

[9]. Alcaraz, I.E;Satorres, S.E.; Lucero, R.M;Puig, S.K. and liu, T.K. Species identification slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens .Bras .J. Microbiol, 2003; 19: 875–879.

[10]. Stepanovic, S.; Jezek, P.; Vukovic, D., Dakic, I. And Petras. Isolation of members of the Staphylococcus scuiri group from urine and their relationship to Urinary tract infection. J. Clin. Microbiol, 2003; 41(11): 5262-5264.

[11]. Guirguitzova, B., D.; Chankova, and B. Zozikov. Staphylococci asuropathogens— frequency of isolation in hospitalized patients and sensitivityto

antimicrobial agents. Ann. Urol. (Paris), 2002; 36: 341–347.

[12]. de Visscher, A.; Haesebrouck, F.; Piepers, S.; Vanderhaeqhen, W.; Supre, K.; Leroy, F.; Van Coillie, E, and De Vliegher S. Assessment of the suitability of mannitol salt agar for growing bovine-associated coagulase-negative staphylococci and its use under field conditions. Res Vet Sci., 2013; 95(2): 347-51.

[13]. Fluck, R.; Wilson, J. and Tomson, C.R. epidemiology of methicillin resistant Staphylococcus aureus bacteraemia amongst patients receiving dialysis for established renal failure in England. Nephron Clin Pract., 2010; 115(1): c261–270.

[14]. Mermel ,L.A; Allon, M. and Bouzam E. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. Clin Infect, 2009; 49(1): 1–45.

[15]. Koksal, F.; Yasar, H. and Samasti M. Antibiotic resistance patterns ofcoagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol. Res., 2009; 164: 404–410.

[16]. Mayhall, C.G. Hospital epidemiology and infection control, 3rd ed. Philadelphia: Lippincott Wiliam and Wilkins, 2004; 495–510.

[17]. Winn, W.C,; Allen, S.D.; Janda, W.M.; Koneman EW ;Procop GW. ;Schreckenberger, P.C.; Woods, G.L.and Koneman's. Color Atlas and Textbook of Diagnostic Microbiology, 2006; 6th ed.Philadelphia.

[18]. Jumaa, F. H., Yass, I. A., Ibrahim, B. K., (2024). Chemistry. Synthesis, Study of Their Liquid Crystal, Laser Properties of New Binary 1,3-Oxazepine-4,7-dione Derivatives and Evaluation of the Antibacterial Activity of Some of Them. 8(9): 3201-3214. *Journal of Global Scientific Research*.