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Antibacterial Activity of Probiotic Lactobacillus acidophilus Against Antibiotic-Resistant Pathogens Isolated from Skin Infection

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ABSTRACT

Background: Probiotics refer to live microorganisms that promote the organism's well-being when consumed in sufficient quantities. *Lactobacillus acidophilus*, a well-established probiotic, is often suggested for its positive impact on health. **Objective:** To investigate the spread of antibiotic resistance and the antibacterial effectiveness of probiotic bacteria against pathogenic bacteria obtained from skin infections. **Methodology:** A total of 40 samples were collected from patients with skin infections in August and October 2017 from two hospitals in Baghdad. The samples originated from patients of various ages and genders. Following standard morphological and biochemical characterization, 69 isolates were identified as *Enterobacter cloacae* (n=12, 17.4%), *Klebsiella pneumoniae* (n=1, 1.4%), *Proteus mirabilis* (n=1, 1.4%), *Pseudomonas aeruginosa* (n=15, 21.7%), and *Staphylococcus aureus* (n=21, 30.4%). Eleven isolates (15.9%) belonged to *Staphylococcus epidermidis*, with the remaining isolates distributed across *Bacillus spp*. (n=6, 8.6%), *Pseudomonas stutzeri* (n=1, 1.4%), and *Enterobacter aerogenes* (n=1, 1.4%). Antibiotic susceptibility testing using nine antibiotics identified 18 isolates resistant to Gentamicin, Cefotaxime, Amikacin, Ceftriaxone, Tobramycin, and Amoxicillin-clavulanic acid. *L. acidophilus*, a potential probiotic, was cultured and evaluated for its inhibitory activity against the isolated skin infection bacteria. **Results:** The results upon evaluating the inhibitory effect of the probiotic *L. acidophilus* against bacteria causing skin infections demonstrated a broad-spectrum inhibitory impact at all tested concentrations against the isolated skin infection pathogens. **Conclusion:** *L. acidophilus*, a probiotic bacterium, demonstrated inhibitory activity against skin infection-causing bacteria.

Key words: bacterial skin infections, multidrug-resistant, probiotic bacteria.
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INTRODUCTION

The emergence of antibiotics over five decades ago marked a paradigm shift in modern medicine. While inherently efficacious against bacteria (1), antibiotic development has become embroiled in a co-evolutionary arms race with intrinsic bacterial mechanisms. This, inadvertently, selects for and promotes the rise of antibiotic resistance. Bacteria have evolved a diverse arsenal of strategies to evade antibiotic lethality and disseminate resistance traits (2), which until recently were thought to impose a significant burden on overall evolutionary fitness (3), allowing susceptible organisms to outcompete their resistant counterparts ultimately. Broadly, these mechanisms can be categorized into innate resistance or, of greater concern to contemporary clinical and agricultural practices, acquired resistance (4) (5). Bacteria exhibit two primary forms of antibiotic resistance: intrinsic and acquired. Innate resistance is an inherent property of particular bacterial species and predates the use of antibiotics as therapeutic

agents. Microorganisms naturally produce antibiotics in their environment to compete with each other. Consequently, they have evolved sophisticated mechanisms to evade the effects of these antimicrobials. This intrinsic resistance explains why some bacteria are naturally resistant to specific antibiotics. In addition to inherent resistance, bacteria possess a remarkable ability to acquire new resistance traits. This can occur through two main mechanisms: Mutations in chromosomal genes. Spontaneous mutations in a bacterium's DNA can lead to the development of resistance. Such mutations might change the target site of the drug such that the drug binds poorly or fails to bind at all. Acquisition of extrinsic genetic elements: Bacteria may become resistant by acquiring genes from other resistant bacteria, and such events occur through the lateral transmission of genetic material. In this case, mobile genetic substances such as plasmids are often involved, which mediate resistance transfer even between unrelated bacterial genera. The rapid acquisition of drug resistance genes, such as those mentioned above, is a serious problem regarding the control of infectious diseases. Antibiotic resistance is the ability to withstand the effects of a drug that would typically eliminate the majority of this kind. This is a genetic adjustment of the bacteria that limits or prevents the drug from being effective against the said bacteria. Many different mechanisms have evolved in bacteria that render antibiotics ineffective, which continue to be a problem for humankind (6). Skin infections constitute a critical domain of interest within the realm of infectious disease management. These infections are caused by a wide range of pathogenic microorganisms, including *Pseudomonas aeruginosa* and *Staphylococcus species* (7). The appearance of these skin pathogens, particularly those with multidrug-resistant (MDR) properties, is alarming as it limits the number of effective means of antimicrobial treatment available. Several significant risk factors create a predisposition for the development of skin infection. Increased risk factors include long periods in hospital, previous courses of antimicrobial therapy, as well as immunosuppression, like in the case of Human Immunodeficiency Virus (HIV) infection (8). Lactic acid bacteria (LAB) include a broad range of Gram-positive, non-spore-forming, and catalase-deficient bacteria from different ecological environments (9). They are classified within the *Lactobacilli ales* order, which encompasses a variety of acid-resistant genera, with *Enterococcus, Streptococcus,* and *Lactobacillus* being among the most extensively researched. Lactic Acid Bacteria (LAB) are also recognized as integral components of the human gut microbiome (10). The designation "probiotic" not only encompasses live microorganisms but also implies that when ingested in adequate amounts, such microorganisms confer health benefits to the host (11). Among these advantageous microbes, *L. acidophilus* is currently the most renowned due to its probiotic properties and is frequently recommended for inclusion in dietary supplements (12).

METHODOLOGY

Samples collection and cultivation

This study investigated skin infections in patients attending Al-Yarmouk Teaching Hospital and Al-Imamein AL-Kadhimein Medical City, Baghdad Governorate, Iraq. Specimens were collected from patients of various ages and genders diagnosed with skin infections between August 2017 and July 2018. Sterile, disposable cotton swabs were used to collect samples from diverse skin infection sites. These samples were then transferred to test tubes containing Stuart transport medium to maintain viability during transport (13). Upon arrival at the College of Biotechnology laboratories, 100 µl aliquots were obtained from each test tube and inoculated onto the Luria-Bertani (LB) broth medium (14). The inoculated cultures were incubated at 37°C for 24 hours to promote bacterial growth. Following incubation, cultures exhibiting heavy growth were subjected to further analysis for the identification of potential pathogens. This process resulted in the isolation of several pathogenic bacterial species, including Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus. Subsequently, the purified isolates were characterized using a combination of morphological, cultural, and biochemical tests for definitive identification (15).

Antibiotic discs

Antibiotic susceptibility testing was performed using antibiotic discs provided by bioanalyse/Turkey (Table 1)

Table (1): Antibiotic discs used in this study

Antibiotics	Symbol	Concentration (µg/disc)
Amikacin	AK	10
Amoxiclar	APC	10
Ceftriaxon	CRO	10
Cefotaxime	CTX	30
Ciprofloxacin	CIP	10
Gentamycin	GN	10
Levofloxacin	LEV	5
Tobramycin	TOB	10
Trimethoprime	TMB	10

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed on the isolated bacteria using the Kirby-Bauer disc diffusion method (16). Following the guidelines established in the 2004 manual on antimicrobial susceptibility testing (reference needed). The results were interpreted using the Clinical Laboratory Standards Institute (CLSI) breakpoints established in 2016 (17). Standardized inocula of each bacterial isolate were prepared by suspending freshly grown cultures in sterile saline solution to achieve a turbidity equivalent to the 0.5 McFarland standards. A sterile cotton swab was then dipped into the adjusted bacterial suspension and used to streak the surface of Mueller-Hinton agar plates. The inoculated plates were left at room temperature for 3-5 minutes to allow for the absorption of excess moisture. Following incubation, the diameters of the clear zones of inhibition surrounding each antibiotic disc were measured in millimeters. These zone diameters were then compared to the established CLSI breakpoints to determine the susceptibility or resistance of each bacterial isolate to the tested antibiotics.

Determination of the inhibitory effect of probiotics against pathogenic bacteria

A culture of Lactobacillus acidophilus, previously isolated at the Biotechnology Research Center, Al-Nahrain University, was cultivated in de Man, Rogosa, and Sharpe (MRS) broth and subsequently inoculated onto MRS agar plates. The cultures were incubated under anaerobic conditions at 37 °C for 24 hours (18). After incubation, the L.acidophilus culture was inoculated into MRS broth and incubated under anaerobic conditions. Following incubation, the culture was centrifuged at 6000 rpm for 10 minutes, and the resulting supernatant (filtrate) was collected. The crude supernatant was then sterilized by membrane filtration, as described by (19).

The antibacterial activity of the L. acidophilus filtrate was assessed using the well diffusion method described by (20). For this purpose, 0.1 ml of broth culture containing 1×10^{8} CFU/ml of each pathogenic bacterial isolate (previously recovered from skin infections) was uniformly spread onto Mueller-Hinton agar plates. Wells of 5 mm diameter were made in the agar using a sterile cork borer, and each well was filled with the sterile L. acidophilus filtrate. After incubation, inhibition zone diameters (mm) were measured and compared to the control well that contained MRS broth only (21). Five milliliters of L. acidophilus culture were concentrated by lyophilization, and then dissolved in D.W. Finally, about 30µl of these concentrated solutions were placed in a well. The inhibition zones for concentrations of L. acidophilus filtrates against the growth of bacterial isolates were measured after 24 hours. The diameters of the inhibition zones surrounding each antibiotic disc were measured.

RESULTS

Collection of samples from skin infections

A total of 40 clinical samples were collected from patients diagnosed with skin infections at Al-Yarmouk Teaching Hospital and Al-Imamein Al-Kadhimein Medical City in Baghdad Governorate, Iraq. The sample collection period spanned from August 2017 to October 2017. Out of the 40 collected samples, only 37 yielded bacterial growth on the LB agar plates. These isolates were subjected to further purification steps to obtain single colony isolates.

Antibiotic susceptibility of pathogenic bacterial isolates

This study employed the Kirby-Bauer disc diffusion method to evaluate the antibiotic susceptibility profiles of bacterial isolates obtained from various skin infections. Among the 69 isolated bacterial pathogens, diverse susceptibility patterns were observed for the nine different antibiotics tested. (data presented in Figure 1).

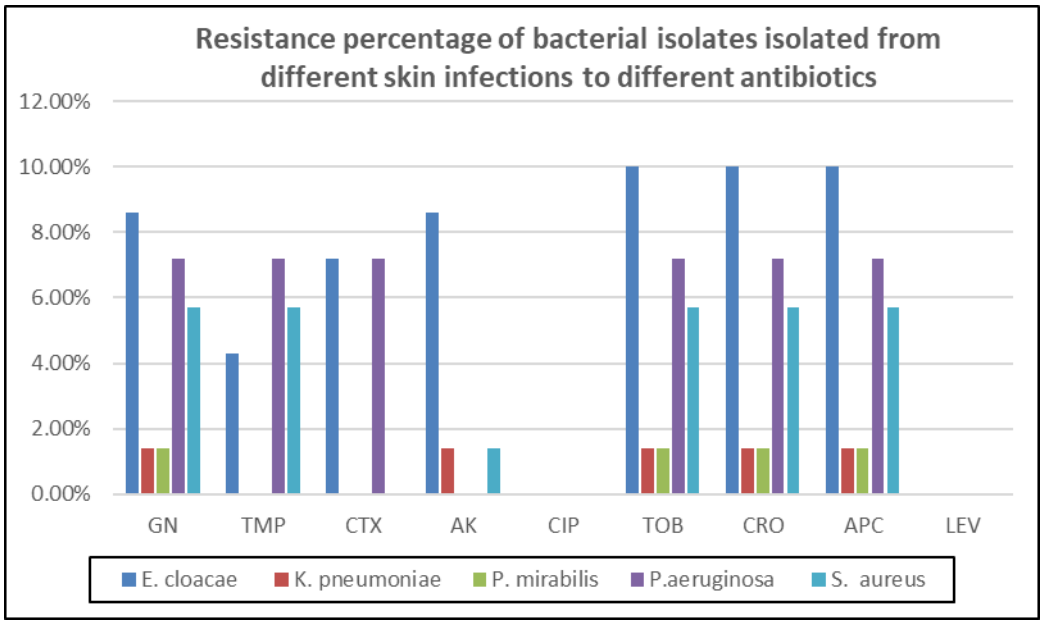

Figure (1): Resistance percentage of bacterial isolates Isolated from different skin infections to different antibiotics

GN: Gentamycin; TMP: Trimethoprim; CTX: Cefotaxime; AK: Amikacin; CIP: Ciprofloxacin; TOB: Tobramycin; CRO: Ceftriaxone; APC: Amoxiclar; LEV: Levofloxacin.

Antibacterial activity of probiotics against pathogenic bacteria *L. acidophilus*

Table (2) demonstrates the antibacterial activity of L. acidophilus filtrate against various bacterial isolates obtained from skin infections, as assessed by the well diffusion method. The results revealed that L. acidophilus exhibited inhibitory effects against all tested pathogens, with varying degrees of sensitivity. The highest zones of inhibition (14 mm) were observed against Enterobacter cloacae and Pseudomonas aeruginosa, indicating strong susceptibility to the probiotic's antimicrobial metabolites. Proteus mirabilis showed moderate sensitivity, with a 13 mm inhibition zone, followed by Klebsiella pneumoniae at 12 mm. Staphylococcus aureus exhibited the lowest susceptibility, showing only a 10 mm zone of inhibition.

Table (2): Inhibitory effect of L. acidophilus probiotic against skin infection isolates.

Bacterial isolates	Inhibition zone (mm)
Enterobacter cloacae	14
Klebsiella pneumoniae	12
Pseudomonas aeruginosa	14
Proteus mirabilus	13
Staphylococcus aureus	10

DISSCUSION

Bacteria possess the ability to develop resistance to antibiotics through two primary mechanisms: intrinsic resistance, inherent to certain bacterial species, and acquired resistance, arising from mutations in chromosomal genes or horizontal gene transfer. Following antibiotic susceptibility testing, eighteen bacterial isolates exhibiting resistance to Gentamicin, Cefotaxime, Amikacin, Ceftriaxone, Tobramycin, and Amoxicillin-clavulanate (Amoxiclar) were selected for further investigation. This selection highlights the concerning global rise of multidrug-resistant (MDR) bacterial infections. (15) Consider extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases to be the major enzymes that cause the phenomenon of multi-drug resistance (MDR) in bacterial populations. In addition, the spread of mechanisms such as altered receptors, loss of antibiotics by enzymic action, and new resistant metabolic pathways greatly aggravates the problem of resistance among Gram-negative bacteria, as evidenced in (22). According to the World Health Organization (WHO), antimicrobial resistance (AMR) is denoted as a microorganism's or microbe's ability to withstand the damaging effects of an antimicrobial agent, such as an antibiotic, to which the microorganism was previously exposed and was prone to. The phenomenon certainly is a common occurrence. However, it has a high rate of occurrence due to the increased abuse of antimicrobials. The European Center for Disease Prevention and Control (ECDC) explains that multidrug resistance (MDR) in certain Gram-negative bacteria, such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter spp., and Proteus spp., is defined when the bacteria feature non-susceptibility against one or more drugs in three or more classes of antimicrobials. These classes of drugs are of utmost importance and vary based on the type of bacterial species that is being targeted (23,24). Amikacin was the least effective of the nine antibiotics tested against all Enterobacteriaceae isolates, with only 73% of isolates showing susceptibility. This was documented in an antibiotic sensitivity profile that showed 73% of isolates were susceptible to amikacin, while resistance rates for ampicillin were (98.5%), ceftriaxone (73.55%), cefotaxime (72%), and ciprofloxacin (58%), 85.5% (171 isolates) of the comprehensive Enterobacteriaceae isolates manifested a multidrug-resistant (MDR) phenotype (25). The development of potent drug resistance within Enterobacteriaceae can be ascribed to several factors, including the alteration of the chromosomal genes, the movement of mobile genetic elements, which can lead to gene transfer, and the acquisition of mobile resistant genes (26). Antibiotic resistance is well known to be spread amongst bacteria with the help of mobile genetic elements such as plasmids, transposons, and integrins. Such elements can take resistance genes from the chromosomes of diverse bacterial species and more often than not transfer them. This class of enzymes aids in the resistance to a wide range of antibiotics that belong to the β-lactam class. The situation is exacerbated by the co-existence of plasmids carrying resistance genes for antibiotics such as quinolone and aminoglycosides, as well as β-lactamase, which enhances the multidrug-resistant characteristic of the pathogenic organisms (27). A global trend of increasing resistance to various anti-pseudomonal drugs, particularly among hospital-acquired strains, has been documented. The isolated P. aeruginosa strains exhibited the highest level of resistance to gentamicin (84%). This finding raises significant concerns, as Gentamicin has traditionally been considered a valuable therapeutic option for P. aeruginosa infections, including those caused by MDR strains. The anti-pseudomonal drugs against P. aeruginosa infections, even against MDR isolates, which is concerning involving 193 P. aeruginosa isolates indicated 79% resistance to Gentamicin, followed by 75% to Ceftriaxone, 73% to Ciprofloxacin, 63% to Ceftriaxon, and 41.5% to Amikacin (28). In the current study, a high prevalence of antimicrobial-resistant Staphylococcus aureus was observed, with a resistance isolation rate of 5.7% from clinical specimens. This finding is significantly lower than those reported in previous studies by (29, 30, 31). Another study (32), revealed high resistance rates to other antibiotics, including Cloxacillin (94.7%) and Cefotaxime (84.2%). The observed prevalence of MDR (100%) in this study is significantly higher compared to the findings of (33,34). When the inhibitory effect of L. acidophilus probiotic against the causative bacteria of skin infections was tested, results showed that an inhibitory effect was recorded at the product or any obtained concentration against all the pathogenic bacteria isolated from skin infections. It is clear that MRS broth is a better stimulator for inhibitory product than on MRS agar and that explained by (19) who recorded that the MRS broth was a stimulated inhibitory effect against Gram-positive bacteria (S. aureus) and Gram-negative bacteria (E. cloacae, K. pneuomonia, P. mirabilis, P. aeroginosa) when inhibition zone diameter ranged between (10-14 mm). Probiotic strains have inhibited pathogenic

bacteria both in vitro and in vivo through several different mechanisms; throughout the production of inhibitory compounds (e.g., bacteriocin), reduction of pH through short chain fatty acid production, which could themselves be directly inhibitory to certain pathogens, competition for nutrients and adhesion sits on the gut wall, modulation of the immune response and regulating colonocyte gene expression (35,36) also noticed the killing action of the bacteriocins as they bind with the cytoplasmic membrane, affect its permeability, and cause death of the sensitive cell.

CONCLUSION

Finally, our work shows that the probiotic strain L. acidophilus shows a notable inhibitory impact against bacteria related to skin diseases. These results imply a possible therapeutic use for L. acidophilus in the treatment and prevention of such diseases.

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النشاط المضاد للبكتيريا لبروبيوتيك لاكتوباسيلاس أسيدوفيلوس ضد العوامل الممرضة المقاومة للمضادات الحيوية المعزولة من عدوى الجلد

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الخلاصة

خلفية البحث: البروبيوتيك تشير إلى الكائنات الحية الدقيقة التي تعزز رفاهية الكائن عند تناولها بكميات كافية. وغالباً ما يُقترح استخدام لاكتوباسيلوس أسيدوفيلوس كبروبيوتيك لتأثيره الإيجابي على الصحة. **الهدف:** التحقيق في انتشار مقاومة المضادات الحيوية وفعالية البروبيوتيك المضادة للبكتيريا ضد البكتيريا الممرضة المستمدة من العدوى الجلدية. **المواد و طرق العمل:** تم جمع مجموعة من 40 عينة من المرضى المصابين بالتهابات جلدية من مستشفيين في بغداد بين آب وتشرين الأول 2017. نشأت العينات من مرضى من مختلف الأعمار والأجناس. بعد التوصيف المورفولوجي والكيميائي القياسي، تم تحديد 69 عزلة كانت من نوعيات البكتيريا التالية: *Enterobacter cloacae* (n=12, 17.4%)، *Klebsiella pneumoniae* (n=1, 1.4%)، *Proteus mirabilis* (n=1, 1.4%)، *Pseudomonas aeruginosa* (n =15, 21.7%)، و *Staphylococcus aureus* (n =21, 30.4%). كانت 11 عزلة (15.9%) تنتمي إلى *Staphylococcus epidermidis*، وكانت العزلات المتبقية موزعة عبر أنواع البكتيريا *Bacillus spp.* (n=6, 8.6%)، *Pseudomonas stutzeri* (n =1, 1.4%)، و *Enterobacter aerogenes* (n =1, 1.4%). أظهرت اختبارات الحساسية للمضادات الحيوية باستخدام تسعة مضادات حيوية وجود 18 عزلة مقاومة Gentamicin, Cefotaxime, Amikacin, Ceftriaxone, Tobramycin, و Amoxicillin-clavulanic acid. تم زرع *Lactobacillus acidophilus*، الذي يعتبر محتملًا كبروبيوتيك، وتقييم نشاطه المثبط ضد بكتيريا العدوى الجلدية المعزولة. **النتائج:** بعد تقييم التأثير المثبط للبكتيريا المحمضة اللاكتوباسيليوس النافعة ضد البكتيريا المسببة للعدوى الجلدية، أظهرت النتائج تأثيرا مثبطا ذو طيف واسع عند جميع التراكيز المختبرة ضد مسببات العدوى الجلدية المعزولة. **الاستنتاج:** خلصت إلى أن اللاكتوباسيليوس النافع، البكتيريا النافعة، أظهر نشاطا مثبطا ضد البكتيريا المسببة للعدوى الجلدية.

الكلمات المفتاحية: العدوى الجلدية البكتيرية ، البكتيريا المقاومة للأدوية المتعددة ، البروبيوتيك.