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Isolation and Identification of Multi-Drug-Resistant Bacteria from the Skin of Diabetic Patients in Mosul, Iraq

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Abstract:

Gramme-positive bacteria were shown to be the primary cause of skin infections associated with diabetes, and they showed varying degrees of resistance to most antibiotics. Additionally, Vancomycin-resistant bacterial isolates were inhibited when a chloromazine minimal inhibitory concentration was employed in conjunction with vancomycin. **Methodology and results:** The current study isolated and identified the bacteria that cause skin infections in diabetic patients. Among the 20 swabs from the skin of diabetic patients, 11(55%) isolates grew and fermented the mannitol salt agar, 7(35%) of them coagulated the plasma in the coagulase test and were positive to catalase test and produced Beta haemolysis on blood agar. The resistance to antibiotics shows all (100%) isolates resistant to Benzylpenicillin and Oxacillin, 5 (71.5%) of isolates *Staphylococcus aureus*, resistant to Tetracycline, 4 (57%) isolates resistant to Erythromycin, 6 (85.7%) isolates resistant to Clindamycin, and 1 (14%) of isolates resistant to Fusidic acid, vancomycin, teicoplanin, and rifampicin, respectively.

Conclusion: According to the study, most skin ulcers in diabetic individuals are caused by gram-positive bacteria, which also exhibit multiple drug resistance. The combination of chlorpromazine and vancomycin also inhibited the bacterial resistance to the antibiotic, improving the likelihood that these ulcers could be treated. It is also advised that more research be done to determine how effective this medication is before applying it to human skin.

Key words: diabetic patients, MDRB, skin inflammation, CPZ, Vitek 2 compact.

Introduction

1.1 Skin Microbiome in Diabetic Patients

The skin is a unique microhabitat and forms a contiguous protective surface covering about 2 m² area (1,2). The skin microbiome is one of the earliest microbial communities to be established on a human's post-birth from maternal and external sources, shaping their lifetime skin health. It comprises a diverse and harmonious microbial community that protects skin homeostasis by defending against pathogen invasion, limiting pathogen colonisation, and regulating host immune

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response (3). However, this symbiosis can become dysregulated owing to intrinsic and extrinsic factors, leading to skin diseases (4,5). Diabetes alters the microbial community of multiple body niches, including the gut, oral cavity, and skin. The skin microbiota in people with diabetes was shown to have unique characteristics compared to non-diabetic individuals, which may affect their skin health (6,7). A comprehensive understanding of skin microbiota could provide insights into the better management of diabetic skin-related conditions. Diabetes-induced changes in the skin microbiota diversity, composition, and relative abundance of certain bacteria have been reported (8). However, it is unclear how glycemic control and factors such as intact or disrupted skin influence the overall skin microbiome in diabetes (9). The skin microbiome is affected by diabetes status, skin integrity, and glycemic control (10). The skin is the largest organ in humans, forming the first line of defense against external insults/pathogens (11). It consists of three main layers, the epidermis, dermis, and hypodermis, with the outer epidermal layer continuously shed, forming a water and protein-rich environment conducive for microbial colonization (12). The microbiota on skin surfaces is dictated by intrinsic factors (such as age, sex, and genetic make-up) and extrinsic factors such as environment, hygiene, and lifestyle (13,14). The skin microbiota is dominated by *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Propionibacterium*, and *Acinetobacter*; however, skin-associated microbes are less diverse compared to other body niches like the gut or oral cavity (15). Due to the risk of comorbidities, compared to non-diabetic patients, diabetic patients have a greater than 83% risk of skin infections, and the incidence of infections increases with age (16). There is a correlation between skin microbiota and the incidence of skin and soft tissue infections in diabetic patients. Efforts were made to collate background information regarding the skin-associated microbes in healthy and diabetic patients, which would be imperative for understanding the complexities of skin-associated microbes and, thereby, the epidemiological, clinical, and microbiological implications of diabetes. Additionally, the isolation and diagnosis techniques of microbes from diabetic and non-diabetic patients have been discussed (17).

1.2 The impact of the skin microbiome on diabetic wound healing

Wounds may become chronic if they are unable to heal within a standard time frame. By 2022, it was anticipated that 220 million individuals would have diabetes worldwide (18). A repercussion of diabetes is the formation of chronic, recalcitrant wounds on the skin; these wounds create a significant health burden due to their recurrent nature and necessitate ongoing medical attention, often leading to the amputation of the limbs (19). The complex metabolic health issues brought on by diabetes hinder the wound's natural ability to recover (20). Wound healing is a highly coordinated multi-stage process, and any dysregulation of the initial inflammatory response and subsequent healing processes modulated by the skin-associated microbiome can severely alter the healing trajectory of the wounds (21). Microbes present on the skin sculpt and actively influence the host's immune responses, and their presence significantly modulates the severity of the wounds and the affected tissue's healing progression (6). As a result of these characteristics, the skin microbiome is now regarded as an important factor in the management of diabetic wounds (22). By utilising high-throughput next-generation amplicon sequencing, proof-of-concept studies were performed to sequence the 16S rRNA gene in all the skin-associated microbes in good and poor healing diabetic wounds (23). A preliminary analysis was also performed to identify specific microbes that either promote or inhibit healing. The crucial necessity for a balanced skin microbiome in promoting healing was supported by this preliminary data analysis (24). In addition, the currently available knowledge on skin microbiomes

was reviewed from the perspective of diabetes-related complications (6). Recent findings related to dysbiosis, or an imbalance in microbial communities, concerning diabetic foot ulcers and other skin-associated conditions were examined. Probiotics, along with other microbiome-modulating therapies, were discussed as a potential avenue of future treatment. The skin microbiome is also in its infancy concerning its clinical relevance; however, there is a concerted effort to understand its role in diabetic wound care (25,26).

Our study aimed to identify and isolate the bacteria that cause skin infections in patients with diabetes, as well as to verify the resistance of these bacteria to antibiotics and to study the synergistic effect of the antipsychotic chlorpromazine with Vancomycin against Vancomycin-resistant bacteria.

2. Methodology

2.1 Isolation Techniques for Microbial Sampling from the Skin

In this study, 20 isolates were taken from the skin of diabetic patients. Samples were taken at the Al-Wafaa Specialised Diabetes Centre. They were obtained from the wounds and ulcers using a sterile cotton swab after a sterile needle was used to scratch the infection site. They were then cultured on blood agar, mannitol salt agar, and MacConkey agar as diagnostic media (27).

2.2. Identification and Diagnosis of Microbes in Skin Samples:

Microbes stranded from skin samples were cultivated in various media to choose distinct colonies. The cultured isolates were then further processed for identification by biochemical, molecular, and phenotypic techniques. Microbial identification is of utmost importance to determine the effective treatment regime against the infecting microbes. It has been reported that identical microbes may display different pathogenicity, necessitating the need for identification (28). A variety of methods are applied for the identification of microbes in infected skin, which involves rapid, culturing, biochemical, and molecular methods (29). In our study, we used a catalase and coagulase test to diagnose the isolates, and then the Vitek 2 compact system was used for the identification process, which begins with a sample that undergoes a series of steps to extract the characteristics of a sample. After incubation, a vial containing the organism is inserted into Vitek 2, where a series of tests are automatically conducted. More than 80 different tests are available to identify a broad range of organisms, with each test providing one specific characteristic. Using a combination of different tests helps to exclude numerous organisms until a unique identification is achieved. The results from these tests are translated into a profile number that represents one organism or a group of closely related organisms. Vitek 2 compares this profile number to a database containing numerous profiles to find the closest match, which is the identified organism (30,31).

Also, Antibiotic susceptibility testing (AST) has been performed to test isolates' susceptibility to the antibiotics that are considered essential for effective infection treatment and controlling antibiotic resistance. The Vitek 2 Compact System automates microbial identification and AST, providing quick results via microdilution techniques. It evaluates susceptibility by exposing microbial strains to antibiotic gradients, using algorithms to interpret data and classify susceptibility based on growth. The minimum inhibitory concentration (MIC) indicates the lowest antibiotic level preventing visible growth, defining susceptibility. Testing over 30 antibiotics across nine pathogen groups, it profiles critical organisms such as Enterobacteriaceae and Pseudomonas. Direct protocols enable testing from positive blood cultures, offering Gram-negative susceptibility results in six hours. The Vitek 2 Compact demonstrates high accuracy compared to traditional methods and significantly reduces turnaround times for bloodstream infection results (32-34).

2.3 Synergism Between Vancomycin (VA) and the Antidepressant Drug Chlorpromazine (CPZ):

A diffusion method was used in his test on Muller-Hinton Agar medium. On the surface of the cultured media, 5 mm pits were made. On one plate the MIC of Vancomycin was made (35,36), and on the other plate 100 microliters of Vancomycin and Chlorpromazine was added (50 microliters each Vancomycin and Chlorpromazine) together with their MICs to study the synergistic effect of the two drugs together, after inoculating the plate with the tested bacteria and the plates were incubated at 37 °C for 24 h. The inhibition diameters were measured around the pits in mm, and the process was repeated twice, and the rate was taken (Egorove, 1985). The interactions between CPZ and Va were calculated using the fractional inhibitory concentration index (FICI) according to the equation FICI = FICA + FICB = A /MICA + B /MIC B. Where A is the antibiotic MIC in the combination, MICA is the antibiotic MIC alone, B is the drug MIC in the combination, and MICB is the drug MIC alone (37).

3. Results

Among the 20 swabs from the skin of diabetic patients, 11 isolates grew and fermented the mannitol salt agar, 7 of them coagulated the plasma in the coagulase test and were positive to catalase test and produced Beta haemolysis on blood agar (Figure 1).

The Vitek 2 compact system ensured the diagnosis, which shows 5 isolates as *Staphylococcus aureus*, one isolate from *Staphylococcus xylosus* and *Staphylococcus gallinarum*, respectively.

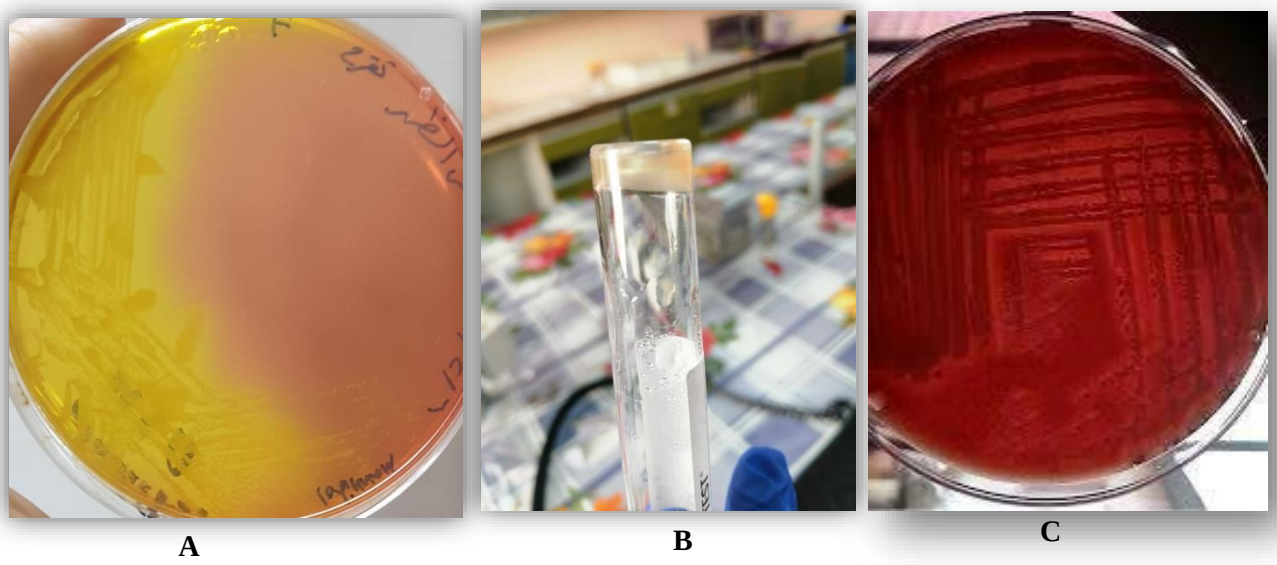
The resistance to antibiotics showed that all isolates resistant to Benzylpencillin and Oxacillin by 100%, Five isolates resistant to Tetracycline by 71.5%, Four isolates resistant to Erythromycin by 57%, Six isolates resistant to Clindamycin by 85.7%, and One sample resistant to Vancomycin, Teicoplanin, Rifampicin as shown in the (Table 2).

The synergistic effect of the drug Chlorpromazine was tested with Vancomycin of one isolate that is resistant to Vancomycin to reduce bacterial resistance, as CPZ significantly reduced the MIC of Vancomycin, where the MIC value of Vancomycin after mixing ranged between (0.015- 8) µg/ml. According to the FICI results, chlorpromazine gave a synergistic effect with vancomycin, as shown in Table 1 and Figure 2.

(Table 1): Inhibition zone diameter of the synergistic effect of vancomycin and chlorpromazine against *Staph. aureus* bacteria.

Isolate No.	VA MIC µg/ml	CPZ MIC µg/ml	VA + CPZ µg/ml
Umayma G	256	64	0.56
Median FICI			**0.56**

VA, Vancomycin, CPZ: Chlorpromazine, FICI: Fractional Inhibitory Concentrations Index. (FICI) values were explained as follows: 1.00–4.00 (indifference); >4.00 (antagonism)



A B C

Figure 1: Growth of Isolates on **A.** Mannitol Salt Agar, **B.** Coagulase test, **C.** Blood agar

Table 2: Antibiotic resistance pattern for isolates understudy. R: resistant. S: sensitive. I: intermediate.

Bacteria / **Antibiotics**	***Staphylococcus aureus*** **(3 isolates)**	***Staphylococcus aureus***	***Staphylococcus aureus***	***Staphylococcus xylosus***
Cefoxitin	**POS+**	**POS+**	**POS+**	**POS+**
Benzyl penicillin	**R.**	**R.**	**R.**	**R.**
Oxacillin	**R.**	**R.**	**R.**	**R.**
Gentamicin	**S.**	**S.**	**S.**	**S.**
Tobramycin	**S.**	**S.**	**S.**	**S.**
Levofloxacin	**S.**	**R.**	**S.**	**S.**
Moxifloxacin	**S.**	**I.**	**S.**	**S.**
Inducible Clindamycin resistance	**POS+**	**POS+**	**NEG-**	**NEG-**
Erythromycin	**R.**	**R.**	**I.**	**S.**
Clindamycin	**R.**	**R.**	**S.**	**R.**
Linezolid	**S.**	**S.**	**S.**	**/**
Teicoplanin	**S.**	**S.**	**R.**	**S.**
Vancomycin	**S.**	**S.**	**R.**	**S.**
Tetracycline	**R.**	**S.**	**R.**	**S.**
Tigecycline	**S.**	**S.**	**S.**	**S.**
Nitrofurantoin	**S.**	**R.**	**S.**	**S.**
Fusidic Acid	**S.**	**S.**	**S.**	**R.**
Rifampicin	**S.**	**R.**	**S.**	**S.**
Trimethoprim / Sulfame thoxazole	**S.**	**S.**	**S.**	**S.**

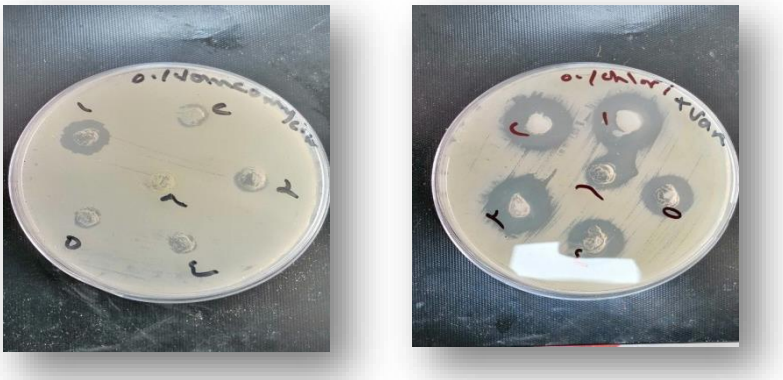


Figure 2: Synergistic Effect of Vancomycin and Chlorpromazine

4. Discussion:

Diabetes mellitus, being a global pandemic, serves as an important cause of susceptibility to bacterial infections because uncontrolled hyperglycaemia is associated with impaired innate and adaptive immune responses that predispose to bacterial infections (38,39). This study found that wound inflammation in diabetics was dominated by positive bacteria. This is due to some variables associated with the disease and its impact on the skin and immune system. Certain gram-positive bacteria, such as *Staphylococcus aureus*, are normally present in the nose or on the skin; these bacteria can readily spread to a wound and result in illness. Because of the effect of circulation and the tissue's poor supply of oxygen and nutrients, wound healing may be delayed in patients with diabetes, increasing the risk of infection (40,41).

The resistance to antibiotics showed that all isolates resistant to Benzylpencillin and Oxacillin by 100%, Five isolates *Staphylococcus aureus*, resistant to Tetracycline by 71.5%, Four isolates resistant to Erythromycin by 57%, Six isolates resistant to clindamycin by 85.7%, and one isolate resistant to Fusidic acid, vancomycin, teicoplanin, and rifampicin (14%), as shown in the (Table 2), and there is evidence to support the idea that diabetics are more susceptible to specific infections due to the attenuation of their immune defences, which can be congenital or attributable to metabolic abnormalities, microangiopathy, and neuropathy. Therefore, it is advised that diabetes patients undergo careful microbiologic surveillance (42). When CPZ demonstrates antibiotic activity against *S. aureus*. It has been shown that this bacterium can be killed by non-toxic CPZ doses below clinical values (43,42), and that using CPZ in combination with beta-lactam antibiotics has a synergistic effect against VRSA and MRSA strains (45,46). Additionally, earlier research employing Transmission Electron Microscopy (TEM) has demonstrated that CPZ impacts the *Staph. aureus* cell envelope. Additionally, at the gene level, CPZ reduced the expression of resistance genes (45,46).

The immune suppression caused by diabetes and the polymicrobial nature of the diabetic infection microenvironment may promote the emergence of novel strains of multidrug-resistant bacterial pathogens (47). The function of the defence mechanism of the autoimmune immune system of diabetic patients is weakened compared with that of normal people, so diabetic patients are prone to concurrent infection, which is not easy to control. In clinical practice, broad-spectrum antibiotics are

often used to control infection, and long-term application of broad-spectrum antibiotics is likely to cause bacterial imbalance and increase drug resistance (48). Also, it prolongs the treatment time. Exposing the wound to the multidrug-resistant bacteria-prone milieu of the hospital increases the likelihood of multidrug-resistant bacterial infection in patients (49).

From the results obtained, we need surveillance of resistant bacteria to reduce the risk of major complications in patients with diabetes mellitus.

Ethical Statement:

This study was conducted on a group of people suffering from diabetes in the city of Mosul after taking their consent to participate in this study, taking into account the complete confidentiality of the patients and not revealing their names in the study.

Conflict of interest: NIL

Funding: NIL

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