

ISOLATION, IDENTIFICATION, AND DETERMINATION OF THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIA ASSOCIATED WITH DIABETIC FOOT ULCER

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ABSTRACT

Objective: This study involved isolating, identifying, and determining the susceptibility patterns of bacteria from diabetic patients who were hospitalized for diabetic foot ulcers.

Methods: The specimen was collected using a deep swabbing approach from the feet of forty hospitalized patients with diabetes. The two sample swabs were delivered to the microbiology laboratory as soon as they were collected. One swab was used for microscopic examinations, and the other was utilized for culture. Three aseptically prepared agars – chocolate, MacConkey, and sheep blood were used for culture. In accordance with accepted clinical standards, the pathogens were identified. By performing the Kirby–Bauer disc diffusion method on Mueller–Hinton Agar medium, the isolates' antibiotic sensitivity patterns were examined.

Results: Twenty-five patients had microorganisms in their foot ulcers, whereas 15 patients had sterile samples (no pathological growth). Gram-negative (10) and positive (15) bacteria were recovered, with some patients having both types. *Pseudomonas aeruginosa* (32%), *Klebsiella* species (8%), and methicillin-resistant (10), sensitive (2), and coagulase-negative (3) strains of *Staphylococcus aureus* were identified.

Conclusion: Imipenem was the antibiotic most sensitive to almost all of the isolates, whereas Penicillin G had more resistance to all of the isolates, and the other antibiotics had more variation. Our findings lead us to recommend that patients with diabetes be empirically given imipenem.

Keywords: Diabetes, Diabetic foot ulcer, Diabetic foot infection, Antibiotic sensitivity.

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INTRODUCTION

Diabetic patients frequently experience costly and difficult foot infections. These not only result in severe morbidities but also represent the majority of diabetes-related hospital admissions and the most prevalent immediate, non-traumatic cause of amputation [1-3]. The cost of treating a diabetic foot ulcer (DFU) is more than twice as high as the cost of treating any other chronic ulcer etiology, according to the most recent data. In addition, 25% of these diabetics will experience foot ulcers at some points in their lifetime. Both persistent wounds and infections have been associated with microbial biofilm. It is anticipated that nearly one in two diabetics who have a DFU will go on to acquire a diabetic foot infection (DFI) [2]. According to estimates, diabetic complications result in lower limb amputations once every 20 s. Complications are frequently experienced by patients with uncontrolled diabetes; among the clinically significant ones are foot ulcers, retinopathy, neuropathy, and macrovascular problems. Foot issues such as foot ulcers are serious public health issues and place a significant load on the health-care system [4]. According to estimates, 15% of diabetics will eventually develop a foot ulcer that becomes osteomyelitis. 20% of diabetes patients experience DFUs, which are caused by peripheral neuropathy, muscle atrophy, foot deformity, and neuropathy fractures. Furthermore, these ulcers might lead to DFI [5-7]. DFI, which may present as ulceration, gangrene, Charcot joint disease, or fractures (Fig. 1), are a major risk factor for amputation. It has been suggested that up to 85% of amputations can be avoided when an effective care plan is adopted. Unfortunately, insufficient training, suboptimal assessment and treatment methods, failure to refer patients appropriately, and poor access to specialist foot care teams hinder the prospects of achieving an optimal outcome [6]. DFIs are usually polymicrobial in nature due to aerobic bacteria (*Staphylococcus* species, *Streptococcus* species, and *Enterobacteriaceae*) and anaerobic

flora (*Bacteroides* species, *Clostridium* species, and *Peptostreptococcus*) and fungi [8]. Proper diabetes control and efficient local wound care are essential components of a comprehensive approach for the successful diagnosis and treatment of patients with DFUs. Preventing infections, using ways to relieve pressure, and restoring pulsatile blood flow [9]. DFIs need to be carefully monitored and managed, ideally by a diverse foot healthcare team. An infectious disease expert, a surgeon, a diabetologist, a radiologist, and a medical microbiologist should ideally be a part of or easily accessible to the team treating these diseases [9]. Compared to wounds from other causes that are not affected by diabetic foot alterations, infection poses the most concern to DFUs. The frequency of infection, associated morbidities, necessity for hospitalization, and length of hospitalization can all be potentially decreased with the best DFI management practices [10].

The present study evaluated the bacteria present in DFU infections and the antibiotic susceptibility patterns of isolates that would be helpful in taking appropriate measures in diagnosis, treatment, and management.

METHODS

Antibiotics

Ampicillin (10 µg/mL), amoxicillin/clavulanic acid (75/10 µg/mL), amikacin (30 µg), ampicillin/sulbactam (10/10 µg/mL), ceftriaxone (30 µg/mL), cefoxitin (30 µg), ciprofloxacin 5 µg, cloxacillin 1 µg, erythromycin (15 µg/mL), gentamycin (10 µg/mL), Teicoplanin 38 µg, ticarcillin/clavulanic acid 75/10 µg, vancomycin (30 µg/mL), penicillin (10 µg/mL), imipenem (10 µg/mL), and oxacillin (1 µg/mL).

Media

Blood agar, Chocolate agar, MacConkey agar, peptone water, triple sugar iron (TSI) agar, citrate agar, Christensen 5 urea agar.



Fig. 1: Diabetic foot ulcer. Amputated toe, (a) the toe had to be amputated due to gangrene. Otherwise, the gangrene would spread to the higher parts of the leg and would require its amputation. Callus, (b) the most frequent cause of ulceration in neuropathic feet is repeated mechanical stress brought on by walking. NFU (neuropathy foot ulcer), (c) NFU is frequently noticeable on the edges of the foot, especially on the side opposing the first and fifth metatarsophalangeal joints



Fig. 2: Sample collection, (a) deep swab technique for collection of wound sample, (b) mechanical debridement. Mechanical debridement of the wound to allow for tissue collection and aeration. Debridement is required to remove tissues and enable hydrotherapy for wounds

Chemicals

Hydrogen peroxide, Kovac's reagent.

Sample collection and processing

Patients who were hospitalized for diabetes had their samples taken. A sterile curette and scalpel were used to clean and debride wounds, and gauze soaked in normal physiological saline was used for cleaning. The specimen was obtained using the deep swab technique (Fig.2). The swabs were placed aseptically in a sterile pack and sealed with sellotape. Moreover, tissue fluids were aspirated using a needle. The samples were immediately sent to the microbiology laboratory for processing.

Gram staining

This method of differential staining is beneficial for bacteriology. It classifies microorganisms into the two clearly identifiable Gram-positive and Gram-negative groups [11]. Briefly, on a dry, transparent glass slide, the swab was applied. Dried by air and fixing with low heat. After that, the area was flooded for 1 min with Gram's Crystal Violet, rinsed with distilled water, and then flooded for 1 min with Gram's iodine. Then, acetone was poured (decolorization step). The sample was stained with Safranin after being rinsed for 1 min. The stains were washed with tap water, then dried before being viewed using an oil immersion objective.

Culture and identification

The specimen's direct Gram-stained smear was evaluated. Blood, chocolate, and MacConkey agar was used to inoculate the specimens. In the laminar airflow, this was carried out aseptically (Fig. 3). The plates were incubated for 24 h at 37°C, and then Gram staining was used to detect the types of isolates and their morphologies. The organisms were further identified by examining their biochemical reactions. Gram-positive cocci isolates were subjected to catalase and coagulase assays, whereas lactose-fermenting Gram-negative bacilli were tested using urease, indole, citrate, and the TSI agar method. The Gram-negative bacteria were further tested for oxidase production [11,12].

Antibiotic susceptibility testing

The Kirby-Bauer disc diffusion method was used for this purpose [13]. We infected the bacterial strain with 3-4 well-isolated colonies and cultured them in peptone water for 3 h. To remove extra fluid, we submerged a sterile cotton swab in the bacterial mixture and rotated it several times while applying force to the tube's interior wall. We applied the swab evenly on the Mueller-Hinton Agar plate's surface before gently pressing the antibiotic discs into place with sterile forceps. Overnight incubation was carried out at 37°C. We classified the observations as sensitive or resistant in the observation sheet.

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta lactamase (ESBL)

MRSA detection

The phenotypic test for the detection of MRSA was done using an Oxacillin (1 g) disc. Resistance to this antibiotic disc was reported as MRSA, whereas those isolates that showed a zone of inhibition were reported as methicillin-sensitive *S. aureus* (MSSA) (Fig. 4) [14].

ESBL detection

The discs ceftriaxone (30 µg), Cefoxitin (30 µg), and imipenem (10 µg) were also used to identify the ESBL. According to the definition, ESBLs are Gram-negative bacteria that cause resistance to extended-spectrum third-generation cephalosporin such as ceftazidime, cefotaxime, and ceftriaxone, as well as monobactams such as aztreonam but not to cephamycins such as cefoxitin and cefotetan or carbapenems (e.g., meropenem or imipenem). They produce the beta lactamase [15]. The aforementioned discs were placed on the Mueller-Hinton agar plate, which had been previously inoculated with the test organism as a lawn. The plates were checked the next day after being incubated at 37°C overnight. The organism was identified as an ESBL because it was sensitive to cefoxitin and imipenem but resistant to ceftriaxone.

RESULTS AND DISCUSSION

Isolation and identification

This study was done at TSRTC Tarnak Hospital and included 39 inpatients admitted to this hospital. Four were female and 35 were male. The age ranges from 40 to 70 years. The patient's symptoms have been present for more than 10 years due to a history of diabetes. The samples were grown overnight on various media. The isolates were stained with Gram staining, and their susceptibility to the aforementioned drugs was evaluated. Antibiotics with a zone of clearance were classified as having sensitive susceptibility patterns, whereas those without one were classified as having resistant susceptibility patterns (Table 1). The antibiogram was calculated based on Table 1. Despite the foot's worsening condition, samples were deemed to have no pathological growth if they failed to exhibit any growth in any of the media. DFUs are more susceptible to bacterial infections that spread rapidly, leading to irreversible tissue damage. Complications usually begin with an unrecognized foot ulcer in a patient with an insensate foot, which gets infected, leading to significant morbidity and lower extremity

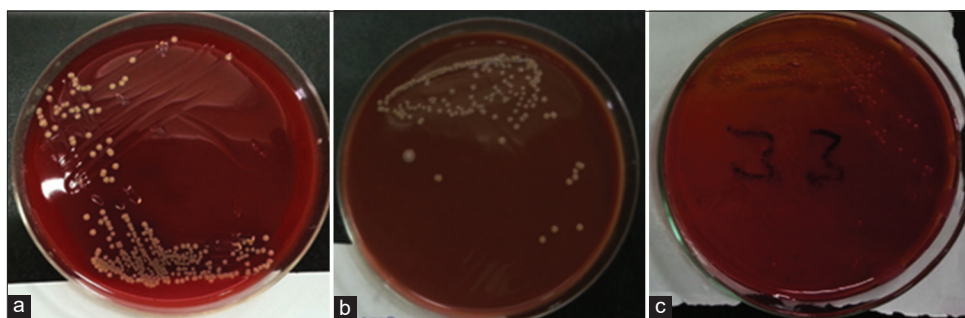


Fig. 3: Colonies in, Blood agar, (a) chocolate agar, (b) MacConkey agar, (c) the three aforementioned mediums were used for the culturing of wound samples. Blood agar is a versatile growth medium for fussy microorganisms. In addition to serving as a differential medium, certain bacteria hemolyse blood, whereas others do not. Chocolate agar is an enhanced medium that contains warm blood and provides a growth advantage for fastidious bacteria. On the other hand, MacConkey media is primarily used for the cultivation and classification of enteric bacteria as either lactose or non-lactose fermenters

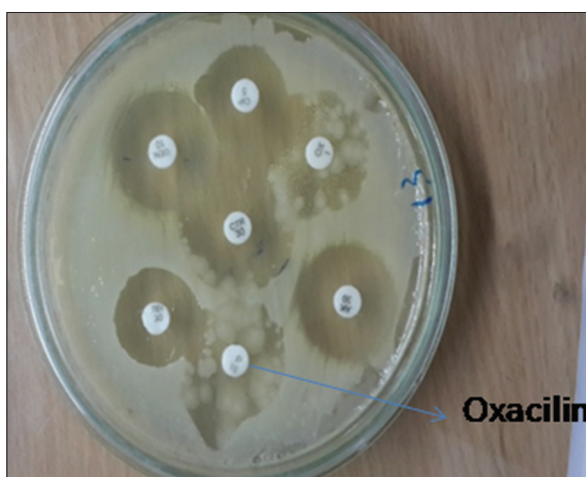


Fig. 4: Oxacillin resistance. Some specimens of *Staphylococcus aureus* were found to be resistant to the antibiotic Oxacillin, and as a result, the organism was referred to as Methicillin-resistant *S. aureus*

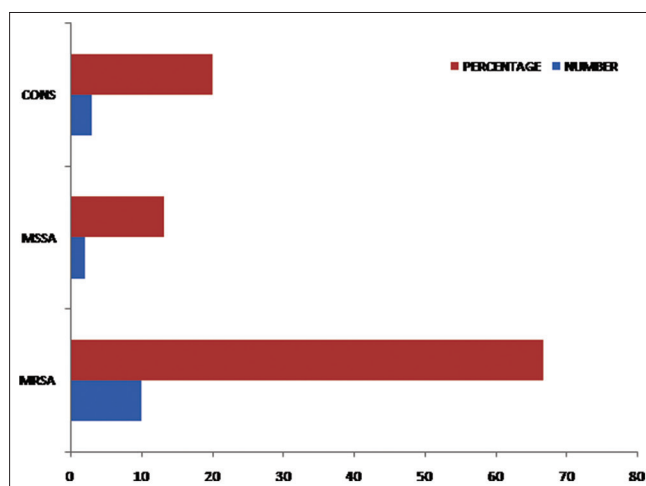


Fig. 6: Number and percentage of Staphylococci. MRSA, methicillin-sensitive *Staphylococcus aureus*, and coagulase-negative staphylococci were used to classify the different types of staphylococci based on how they coagulate rabbit plasma. 10 MRSA (66.7%), MSSA 2 (13.3%), and CoNS 3 (20%) were detected

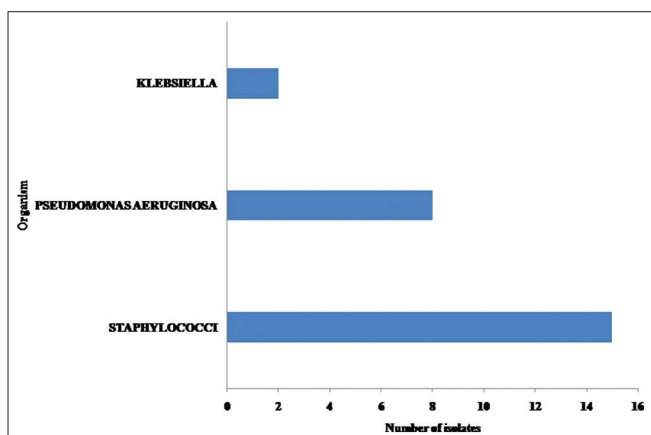


Fig. 5: Number of diabetic foot ulcer pathogens isolated

amputations. We did not observe any cases of amputation during our study period. Patterns of microbial infection are not consistent in patients with DFI, and therefore repeated evaluation of microbial characteristics and their antibiotic sensitivity is necessary for the selection of appropriate antibiotics. The progression of infection in the diabetic foot is a result of suppressed immune status, delayed diagnosis, underestimation of the extent of infection, or suboptimal (if not inappropriate) antimicrobial therapy. We observed that Gram-positive

infections were more common in the studied population, in contrast to other studies that were done in North India. There was a prevalence of MDROs in this study.

The identification of the organisms was done following criteria in Table 2, before Gram staining. Twenty-five organisms were isolated, and 15 of the cultures were sterile. Out of the 25 isolates, 40% (10) were Gram-negative, comprising *Klebsiella* spp. (2) and *Pseudomonas aeruginosa* (8) and 60% (15) were Gram-positive cocci (*Staphylococcus* spp.) (Fig. 5). The organisms that were isolated are staphylococci, among which 10 (66.7%) were MRSA, 13.3% were MSSA, and 20% were coagulase-negative staphylococci (CoNS) (Fig. 6). Among the Gram-negative organisms, pseudomonas accounted for 32% of the total isolates and *Klebsiella* 8%. Only two *Klebsiella* species were identified, of which 50% (or 1) were identified as ESBLs.

Antibiogram pattern of the isolates

The pattern of resistance is shown in Table 3. Amikacin was 100% sensitive to MSSA but resistant to 20% MRSA, 33% CoNS, 50% *Klebsiella* spp., and 62.5% *P. aeruginosa*. Amoxycyclavonic acid was resistant to 12.5% of *P. aeruginosa*. 100% CoNS, 100% *Klebsiella*, and 50% MRSA 90% of MRSA, 50% of MSSA, 66% of CoNS, 100% of *Klebsiella*, and 62.5% of *P. aeruginosa* were resistant to ampicillin. 20% of MRSA and 100% of CoNS, 50% of *Klebsiella* spp., and 12.5% of *P. aeruginosa* were resistant to ceftriaxone; however, 100% of MSSA was sensitive. Cefoxitin was 100% sensitive to MSSA but 60% sensitive to MRSA,

Table 1: Patient entry, date, age, sex. Organism isolated and susceptibility pattern

Patient No.	Age	Sex	Name of the organism	Susceptibility pattern	
				Sensitive	Resistance
1	49	M	No Pathological Growth	-	-
2	59	M	No Pathological Growth	-	-
3	50	M	<i>Staphylococcus aureus</i> (MSSA)	Ampicillin Gentamicin Amoxyclav Vancomycin Imipenem Oxacillin Ceftriaxone Cefoxitin	Penicillin G Ticarcillin/clav
4	54	M	<i>Staphylococcus aureus</i> (MSSA)	Amikacin Amoxyclav Vancomycin Imipenem Oxacillin Ceftriaxone Cefoxitin Teicoplanin Ciprofloxacin	Penicillin G Ampicillin Ticarcillin/Clav
5	55	M	No pathological growth	-	-
6	44	F	No pathological growth	-	-
7	58	M	No pathological growth	-	-
8	54	M	No pathological growth	-	-
9	50	M	<i>Staphylococcus aureus</i> (MRSA)	Imipenem Vancomycin Ampicillin Amikacin Teicoplanin Gentamicin Ciprofloxacin Ceftriaxone	Cefoxitin Ticarcillin/Clav Penicillin G Oxacillin
10	55	M	No pathological growth	-	-
11	47	M	No pathological growth	-	-
12	51	M	No pathological growth	-	-
13	59	F	<i>Staphylococcus aureus</i> (MRSA)	Vancomycin Erythromycin Teicoplanin Gentamicin Amikacin Ciprofloxacin Ceftriaxone Imipenem	Ampicillin Cefoxitin Oxacillin Penicillin Ticarcillin/clav
14	52	M	Coagulase negative staphylococci	Amikacin Imipenem	Gentamicin Teicoplanin Ampicillin Vancomycin Teicoplanin Ciprofloxacin Ceftriaxone
15	70	F	<i>Staphylococcus aureus</i> (MRSA)	Imipenem Gentamicin Vancomycin	Oxacillin Penicillin Ampicillin Erythromycin Teicoplanin Ciprofloxacin Ceftriaxone Amikacin Ticarcillin/Clav Cefoxitin
16	60	M	No pathological growth	-	-
17	46	M	Coagulase negative staphylococci	Imipenem Gentamicin Ciprofloxacin Teicoplanin Vancomycin	Penicillin Ampicillin Oxacillin Cefoxitin Erythromycin

(Contd...)

Table 1: (Continued)

Patient No.	Age	Sex	Name of the organism	Susceptibility pattern	
				Sensitive	Resistance
18	42	M	No pathological growth	-	Amikacin Ticarcillin/Clav Ceftriaxone
19	46	M	Coagulase negative staphylococci	Imipenem Gentamicin Ciprofloxacin Teicoplanin Vancomycin	Penicillin Ampicillin Oxacillin Cefoxitin Erythromycin Amikacin Ticarcillin/Clav Ceftriaxone
20	63	M	CoNS	Imipenem Erythromycin Cefoxitin Amikacin Ceftriaxone Vancomycin	Oxacillin Gentamicin Ampicillin Penicillin Ticarcillin/Clav Ciprofloxacin
21.	53	M	<i>Pseudomonas aeruginosa</i>	Amikacin Gentamicin Vancomycin Imipenem Ciprofloxacin	Ampicillin Amoxyclav Cefoxitin Ceftriaxone Ticarcillin/Clav Oxacillin Penicillin Teicoplanin
22	41	M	No Pathological Growth	-	-
23	52	M	Klebsiella spp.	Gentamicin Vancomycin Amikacin Imipenem Cefoxitin	Ceftriaxone Ticarcillin/Clav Penicillin Amoxyclav Oxacillin Ciprofloxacin Teicoplanin Erythromycin
24	70	M	No pathological Growth	-	-
25	54	M	<i>Pseudomonas aeruginosa</i>	Imipenem Teicoplanin Gentamicin Ceftriaxone	Teicoplanin Oxacillin Cefoxitin Penicillin Amikacin Ciprofloxacin Erythromycin Ampicillin Amoxyclav Vancomycin
26	56	M	<i>Pseudomonas aeruginosa</i>	Cefoxitin gentamicin, Amikacin, amoxyclav	Erythromycin, vancomycin, ampicillin, Ceftriaxone Oxacillin, Ticarcillin/clav Teicoplanin, penicillin, ciprofloxacin
27	52	M	<i>Pseudomonas aeruginosa</i>	Erythromycin, Vancomycin Teicoplanin Ciprofloxacin	Oxacillin ampicillin, gentamicin, Imipenem, Ceftriaxone, amoxyclav, Teicoplanin, Cefoxitin Amikacin

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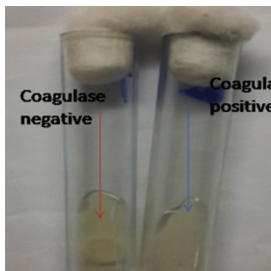
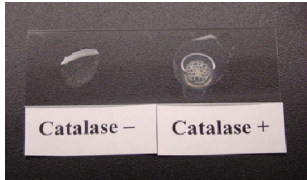
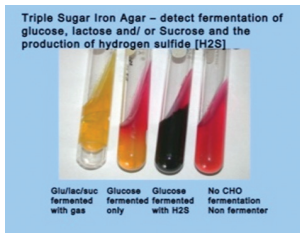
Patient No.	Age	Sex	Name of the organism	Susceptibility pattern	
				Sensitive	Resistance
28	40	F	No pathological growth	-	-
29	52	F	<i>Staphylococcus aureus</i> (MRSA)	Amikacin, Ciprofloxacin Gentamicin Ceftriaxone, Vancomycin Imipenem, Amoxyclav Ticarcillin/clav	Oxacillin, Ampicillin, Teicoplanin Cefoxitin Erythromycin
30	54	M	<i>Pseudomonas aeruginosa</i>	Imipenem	Amikacin Cefoxitin Ampicillin Penicillin Teicoplanin Ciprofloxacin Ticarcillin/clav Vancomycin Amoxyclav Gentamicin Oxacillin
31	48	M	<i>Staphylococcus aureus</i> (MRSA)	Teicoplanin Amikacin Imipenem Cefoxitin Ticarcillin/clav Amoxyclav Vancomycin Ciprofloxacin Gentamicin	Ampicillin Penicillin Oxacillin
32	53	M	<i>Pseudomonas aeruginosa</i>	Imipenem Amikacin Gentamicin Vancomycin	Ampicillin Erythromycin Cefoxitin Amoxyclav Oxacillin Penicillin Ticarcillin/clav Ciprofloxacin Amoxyclav Ticarcillin/Clav
33	55	M	Klebsiella	Vancomycin Teicoplanin Ceftriaxone Imipenem Gentamicin Ciprofloxacin Amikacin	Ampicillin Penicillin Ticarcillin/Clav
34	47	M	<i>Staphylococcus aureus</i> (MRSA)	Ceftriaxone Erythromycin Amoxyclav Teicoplanin Vancomycin Cefoxitin Imipenem	Ticarcillin/clav Amikacin Oxacillin Penicillin Ciprofloxacin ampicillin
35	52	M	<i>Pseudomonas aeruginosa</i>	Imipenem Imipenem Ciprofloxacin	Ampicillin Penicillin Gentamicin Oxacillin Vancomycin Teicoplanin Ceftriaxone Amoxyclav Teicoplanin Amikacin Erythromycin

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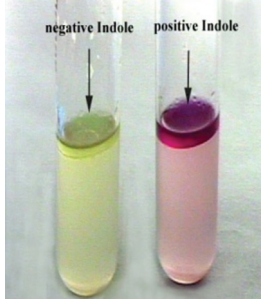
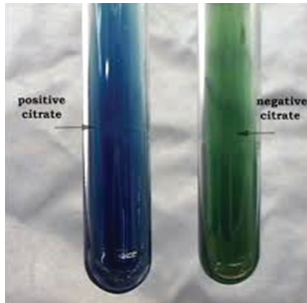
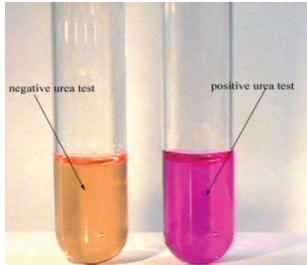
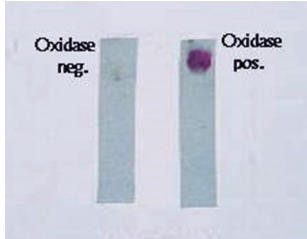
Patient No.	Age	Sex	Name of the organism	Susceptibility pattern	
				Sensitive	Resistance
36	47	M	<i>Staphylococcus aureus</i> (MRSA)	Imipenem Vancomycin Teicoplanin Ceftriaxone Cefoxitin	Gentamicin Erythromycin Amikacin Ciprofloxacin Amoxyclav Oxacillin Teicoplanin Ampicillin
37	52	M	<i>Pseudomonas aeruginosa</i>	Imipenem Ciprofloxacin Teicoplanin	penicillin Penicillin Oxacillin Amikacin Cefoxitin Ampicillin Gentamicin Teicoplanin Vancomycin Ceftriaxone Erythromycin amoxyclav Oxacillin
38	53	M	<i>Staphylococcus aureus</i> (MRSA)	Amikacin Ceftriaxone Teicoplanin Cefoxitin Ciprofloxacin Imipenem Erythromycin Gentamicin	Ampicillin Penicillin Teicoplanin amoxyclav
39	52	M	No pathological growth	-	-

Table 2: Biochemical tests for identification of clinical isolates from diabetic feet

Test	Principle	Observations
Coagulase	One distinctive trait of pathogenic <i>Staphylococcus</i> is the enzyme coagulase, which is generated by a small number of <i>Staphylococcus</i> species. The bacteria may “wall” off its infection from the host’s defense mechanisms very successfully thanks to the enzyme that causes blood to coagulate	
Catalase	The catalase enzyme is produced by several microorganisms. The enzyme decomposes hydrogen peroxide into water and oxygen. Hydrogen peroxide, a byproduct of aerobic respiration, can destroy bacteria if it accumulates inside their cells. Catalase breaks down the hydrogen peroxide there before it damages the bacterial cell	
TSI	Compare and contrast a number of <i>Enterobacteriaceae</i> genera or subgroups. Based on their capacity to produce hydrogen sulfide and how they digest carbohydrates, different types of gut bacteria can be identified. H2S will generate an insoluble black precipitate, which is an indicator of its presence. The indicator used to find this H2S is ferrous sulfate.	

(Contd...)

Table 2: (Continued)

Test	Principle	Observations
Indole test	An amino acid called tryptophan can be found in the peptone water of the culture medium, and when the enzyme tryptophanase reacts with it, it is transformed into indole, skatole, and indole acetic acid. Only a few bacteria can generate indole. Aldehydes and indole interact to create a crimson product. The test's aldehyde is para-dimethylamino benzaldehyde.	
Citrate utilization	Certain microorganisms can obtain all of their carbon from citrate and use it as their exclusive source of energy. Movement of citrate within the cell is facilitated by citrate permease. Oxaloacetic acid and acetate are produced by the action of the enzyme citrase on citrate. Afterwards, pyruvic acid and carbon dioxide are produced by the enzymatic conversion of these compounds. As a result of the CO ₂ produced during this reaction combining with the salt and water to create sodium carbonate, an alkaline product, the medium becomes alkaline. The indicator bromothymol blue, which is present in the medium, shifts from green at pH 6.9 to deep Prussian blue at pH 7.6 in the presence of sodium carbonate.	
Urease test	Urea is a carbonic acid diamide. The bacterial and fungal enzyme urease hydrolyzes urea and emits ammonia and carbon dioxide. The alkaline ammonium carbonate that is produced when ammonia interacts with solution raises the pH of the medium. The existence of urease activity is indicated by the medium's incorporation of phenol red, which turns from yellow to red at an alkaline pH.	
Oxidase test	The iron-containing hemoproteins known as cytochrome serve as the final link in the chain of aerobic respiration by converting electrons (from hydrogen) to oxygen with the creation of water. One of the reagent colors used in the cytochrome oxidase test is p-phenylene diamine dihydrochloride, which serves as a synthetic alternative to oxygen as an electron acceptor. Indophenol blue, a substance with a purplish blue color, is produced when this enzyme oxidizes the chemical N-N tetramethylpara-phenylenediamine hydrochloride, which is a color less chemical in reduced form.	

TSI: Triple sugar iron

Table 3: Percentage of antibiotic resistance pattern

Antibiotic	MRSA	MSSA	CoNS	Klebsiella	Pseudomonas
Amikacin	20	0	33	50	62.5
Amoxyclav	50	0	100	100	12.5
Ampicillin	90	50	66	100	62.5
Ceftriaxone	20	0	100	50	12.5
Cefoxitin	60	0	66	100	12.5
Erythromycin	100	0	66	100	87.5
Gentamicin	10	50	50	50	50
Imipenem	10	0	0	0	12.5
Oxacillin	70	100	33	100	100
Penicillin G	100	100	100	100	100
Teicoplanin	40	0	33	100	100
Ticarclillin/clav	10	0	66	50	75
Vancomycin	10	50	33	50	37.5
Ciprofloxacin	40	0	66	50	37.5

MRSA (methicillin resistant *Staphylococcus aureus*), MSSA (methicillin sensitive *Staphylococcus aureus*), CoNS (Coagulase-negative staphylococci). Percentage sensitivity is 100% min percent resistance.0% resistance is same as 100% sensitivity

66% sensitive to CoNS, 100% sensitive to *Klebsiella* spp., and 12.5% sensitive to *P. aeruginosa*. Erythromycin was 100% sensitive to MSSA but 100% resistant to MRSA, 66% CoNS, 100% *Klebsiella* spp., and 87.5% *P. aeruginosa*. 10% MRSA, 50% MSSA, 50% CoNS, 50% *Klebsiella* spp., and 50% *P. aeruginosa* were resistant to gentamicin. Imipenem was resistant to 10% of MRSA and 12% of *P. aeruginosa* but sensitive to 100% of CoNS, 100% of *Klebsiella* spp., and 100% of MSSA. 70% MRSA, 100% MSSA, 33% CoNS, 100% *Klebsiella* spp., and 100% *P. aeruginosa* were resistant to oxacillin. Penicillin G was ineffective against 100% of MSSA, 100% *P. aeruginosa*, 100% MRSA, 100% *Klebsiella* spp., 100% CoNS, and 100% MSSA. Teicoplanin was 100% sensitive to MSSA and 60% sensitive to MRSA, 33% resistant to CoNS, 100% resistant to *Klebsiella* spp., and 100% resistant to *P. Aeruginosa*. 10% MRSA, 50% MSSA, 66% CoNS, 50% *Klebsiella* spp., and 75% *P. aeruginosa* were resistant to Ticarcillin-clavulanic acid. Vancomycin was 50% sensitive to MSSA but resistant to 10% MRSA, 33% CoNS, 50% *Klebsiella* spp., and 37.5% *P. aeruginosa*. Ciprofloxacin was 100% sensitive to MSSA but resistant to 40% of MRSA, 66% of CoNS, 50% of *Klebsiella* spp., and 37.5% of *P. aeruginosa*.

CONCLUSION

The prevalence of Gram-positive infection was higher in diabetic foot patients from our study. There were rare cases of polymicrobial infection. Imipenem showed the highest sensitivity and it may be started empirically based on the clinical characteristics of infection and can be changed subsequent to learning the results from a definitive bacteriological study. Sometimes, culture reports are negative despite the deteriorating condition of the wound and other clinical findings. In such cases, application of molecular techniques may help to identify microorganisms in the diabetic foot wound and to choose suitable antibiotics against them.

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CONFLICT OF INTEREST

No conflict of interest.

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