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BACTERIAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY TEST AMONG DIABETES MELLITUS PATIENTS WITH GANGRENE IN SURABAYA

PROFIL BAKTERI DAN UJI KERENTANAN ANTIBIOTIK PADA PASIEN DIABETES MELITUS DENGAN GANGREN DI SURABAYA

Original Research Article
Penelitian

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A B S T R A C T

Background: *Gangrene is a severe complication of damaged tissue that can occur in people with Diabetes Mellitus (DM), putting them at risk for bacterial infection. A pus culture can show diabetic gangrene patients' infecting bacteria.* ***Purpose:*** *Determine the prevalence of infection-causing bacteria and antibiotic sensitivity tests in diabetic gangrene patients at Haji Regional General Hospital, East Java Province, for January - December 2021.* ***Method:*** *The method used in this study is observational analytical cross-sectional, which is based on secondary data and is analyzed using the percentage formula and Chi-Square test.* ***Result:*** *The data obtained from 39 patients revealed 29 (74.4%) positive patients for bacterial infection. The Gram-negative bacteria was found to cause infection more frequently (72.41%) than the Gram-positive bacteria (27.59%). The prevalence of Gram-negative bacteria species most frequently from Escherichia coli (ESBL) 13.79% (4/29), Klebsiella pneumoniae 10.35% (3/29), Proteus mirabilis 10.35% (3/29). While the dominant Gram-positive bacteria a Methicillin-Resistant Staphylococcus aureus (MRSA) 10.35% (3/29). The antibiotic sensitivity test showed that Gram-negative group were susceptible to ertapenem, meropenem, amikacin, gentamicin, and piperacillin tazobactam while resistant to ampicillin and cefazolin. The antibiotic sensitivity tests showed that the Gram-positive group was susceptible to linezolid, vancomycin, and tigecycline while resistant to tetracycline and ciprofloxacin.* ***Conclusion:*** *It is important to screen the bacterial profile causing gangrene and their antibiotic susceptibility pattern in DM patients in order to give proper treatment to DM patients.*

A B S T R A K

Latar belakang: Gangren merupakan komplikasi serius berupa jaringan rusak yang dapat terjadi pada penderita Diabetes Melitus (DM) dan sangat berisiko mengalami infeksi bakteri. Pemeriksaan menggunakan kultur pus dapat menunjukkan bakteri penyebab infeksi pada penderita gangren diabetik. **Tujuan:** Mengetahui prevalensi bakteri penyebab infeksi dan uji kepekaan antibiotik pada penderita gangren diabetik di Rumah Sakit Umum Daerah Haji Provinsi Jawa Timur periode Januari - Desember 2021. **Metode:** Metode yang digunakan pada penelitian adalah *observational analytical cross-sectional* dari data sekunder, yang dianalisis dengan rumus presentase dan *Chi-square*. **Hasil:** Diperoleh dari total 39 pasien yaitu 29 (74,4%) pasien positif terinfeksi bakteri. Bakteri penyebab infeksi tertinggi oleh kelompok Gram-negatif yaitu 72,41% (21/29), dan bakteri Gram-positif yaitu 27,59 % (8/29). Prevalensi spesies bakteri Gram-negatif yang dominan oleh *Escherichia coli* (ESBL) 13,79% (4/29), *Klebsiella pneumoniae* 10,35% (3/29), dan *Proteus mirabilis* 10,35% (3/29). Sedangkan pada Gram-positif dominan oleh *Methicilin-Resistant Staphylococcus aureus* (MRSA) 10,35% (3/29). Uji kepekaan antibiotik yang diperoleh berdasarkan kelompok Gram-negatif sensitif adalah ertapenem, meropenem, amikacin, gentamicin, piperacillin tazobactam serta resistan terhadap ampicillin dan cefazoline. Uji kepekaan antibiotik kelompok Gram-positif sensitif terhadap linezolid, vancomycin, dan tigecycline. Sedangkan antibiotik yang resistan yaitu tetracycline dan ciprofloxacin. **Kesimpulan:** Perlunya skrining untuk melihat profil bakteri penyebab gangrene beserta pola kerentanannya terhadap antibiotik pada pasien DM sehingga dapat memberikan pengobatan yang tepat pada pasien DM.

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INTRODUCTION

Diabetes Mellitus (DM) is a rapidly spreading health problem. In 2045, 700 million adults worldwide are predicted to have the disease (International Diabetes Federation (IDF), 2019). Diabetes patients commonly experience foot complications. Diabetes patients frequently experience foot problems, with foot ulcers being one of the more devastating effects. If *Diabetic Foot Infections* (DFI) are not promptly and properly treated, these ulcers frequently get infected and can result in septic gangrene and amputation. Amputation is possible with *Diabetic Foot Ulcers* (DFUs), a severe consequence of diabetes mellitus (Apelqvist *et al*., 2011; Spreen *et al*., 2016). Studies have indicated that diabetes persons are eight times more likely to experience a vascular lower limb amputation at or near the trans-metatarsal level than nondiabetic individuals under the age of 45 (Johannesson *et al*., 2009).

The most common way to define diabetic foot infections is as an inflammatory reaction and tissue harm brought on by an interaction between the host and microbial pathogens (Williams *et al*., 2004). Reduced peripheral circulation, inflammation, and infection have all been put out as potential causes of gangrene, though these theories have been contested (Gershater *et al*., 2009; Schaper *et al*., 2016). Although a few studies have described certain filamentous fungi and yeasts as the etiological agents of diabetic foot infections, the majority of infections in the diabetic foot are of aerobic and anaerobic bacterial origin and, in most cases, polymicrobial (Akhi *et al*., 2015; Kaur *et al*., 2022).

Gram-positive bacteria like *Staphylococcus aureus* and *Enterococcus faecalis,* together with Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, were shown to be the most prevalent flora of diabetic foot infections, according to earlier investigations (Alhubail *et al*., 2020; Heravi *et al*., 2019). With the advancement of diabetes epidemiology and modifications in the use of antimicrobial medications, the bacterial spectrum of diabetic foot infections has changed significantly in recent years (Chen *et al*., 2017; Saltoglu *et al*., 2018). It is crucial to concentrate on evaluating the risk factors of multi-drug resistant bacterial infections in order to find a more effective treatment because the increasingly severe form of prevention and treatment of diabetic foot ulcer infections is associated with a high rate of detection of multi-drug resistant bacteria (Agbi *et al*., 2017; Belefquih *et al*., 2016). Commonly in diabetic foot infection, we can find *Methicillin-resistant Staphylococcus aureus* (MRSA), *Vancomycin-Resistant Enterococcus* (VRE), *Enterobacteriaceae* that produce ultra-broad spectrum-lactamases (ESBLs), such as *Escherichia coli* and *Klebsiella pneumoniae*, *carbapenem-resistant Enterobacteriaceae*, *Multidrug-Resistant Pseudomonas aeruginosa* (MDR-PA) (Yan *et al*., 2022). Thus, this study aims to list bacterial profiles causing gangrene (wet gangrene) among diabetic patients in Surabaya.

MATERIAL AND METHOD

Study site and population

A descriptive observational analysis study was carried out from January to December 2021 at East Java Province Government Hospital, Surabaya Indonesia. The ethical clearance was obtained from ethical committee of this hospital (Number 070/1024/102.10/2022). This hospital is a government building that also act as a teaching facility, with at least 200 beds, this hospital is classified as a type B hospital in Indonesia, and patients with National Health Insurance coverage are welcome to receive care there. Additionally, several district hospitals in Surabaya and the province of East Java used this hospital as their model facility. This hospital offers seventeen different specialties, including pediatrics, anesthesia, dental specialties, internal medicine, radiology, and others.

Data and specimen collection

Wound bed swab sample was collected from 39 DM patients with gangrene. Levine's technique was used to collect surface swabs, which involves rotating a wound swab over a 1 cm^2 area of the wound for 5 seconds while applying enough pressure to draw fluid from the inner portion of the wound bed. The swab was then transported with Amies nutritional growth medium (Labware, USA). Within 20 minutes, the specimens had been packaged in sterile transport containers and delivered to the microbiology facility for aerobic culturing. Samples were then processed to the Microbiology Laboratory Department.

Culture and identification techniques

Swab samples were inoculated onto 5% blood agar plates, MacConkey agar (Oxoid Ltd, Basingstoke, UK). Incubation was carried out overnight under aerobic conditions at 37°C. The next day, the visible and pure colonies were collected and transferred to an inoculum tube containing 3 ml NaCl 0.45% pH 5.0. In order to preserve pure cultures, subcultures of many distinct colonies were carried out in cultures yielding more than one bacterium. The suspension density was then checked with a densitometer (Grant Instruments, UK). The measured density should show 0.5 - 0.63 McFarland.

The inoculum tube was then placed in front of the ID card, and another tube was placed in front of the AST card and then loaded into the VITEK2 system. The VITEK® 2 device (bioMérieux, USA) was utilized to identify the isolates using conventional bacteriological techniques and biochemical testing.

Antimicrobial susceptibility testing

Antibiotics sensitivity testing was performed on sensitivity test agar using VITEK®2 apparatus (bioMérieux, USA) in accordance with the National Committee for Clinical Laboratory Standards. Antibiotic class penicillin (ampicillin); penicillin and a beta-lactamase inhibitor (ampicillin sulbactam, piperacillin/ tazobactam), class cephalosporins (cefazolin, ceftazidime, ceftriaxone, cefepime), class carbapenems (ertapenem, meropenem), class beta-lactamase inhibitor (aztreonam), class nitrofurantoin (nitrofurantoin), class aminoglycosides (amikacin, gentamicin), class fluoroquinolone (ciprofloxacin), and class glycylcycline (tigecycline) were used for Gram-negative bacteria. Antibiotic class penicillin (ampicillin, oxacillin, benzylpenicillin); class aminoglycosides (gentamicin); class glycylcycline (tigecycline); class sulfonamides (trimethoprim/sulfamethoxazole); class tetracycline (tetracycline); class fluoroquinolones (moxifloxacin, levofloxacin, ciprofloxacin); class lincomycin (clindamycin); class oxazolidinone (linezolid), class glycopeptide (vancomycin), class macrolides (erythromycin); class nitrofurantoin (nitrofurantoin) and class rifampicin (rifampicin) were used for Gram-positive bacteria. Antibiotics were classified using the 2019 WHO *Access, Watch, Reserve* (AwaRe) classification of antibiotics for evaluation and monitoring of use. Results were classified as either resistant (R) or sensitive (S) to the tested antibiotics using the interpretive guidelines given by the *Clinical and Laboratory Standards Institute* (CLSI). The tested antibiotics can still be utilized for therapy because of the sensitive results. Since there are bacterial resistances to the antibiotics being tested in this investigation, the readings utilizing this technique (S or R type) reveal this. Multi-drug resistance was defined as bacterial isolates resistant to three or more antimicrobials from various structural classes (MDR).

Statistical analysis

The results obtained in this study are a number of bacteria based on species identification and results of antibiotic susceptibility tests. Categorical data is presented in the form of frequency and percentage.

RESULT

Our results show that among 39 DM patients with gangrene, 19 patients (48.3%) were male, and 20 patients (51.7%) were female. Among those patients, 76.9% (30/39 patients) were included in productive age category (45-59 years), while 7.7% (3/39 patients) were categorized as young adult or 25-44 years old and 15.4% (6/39 patients) were included in elderly category or above 65 years old (Table 1). Pus culture showed microbial growth in 29 patients (74.4%), while 10 patients (25.6%) showed no microbial growth.

Wound swab culture showed the appearance of *Microbial Drug Resistance Organisms* (MDRO), which is *Escherichia coli*-extended-spectrum beta-lactamase (ESBL) in 4 patients (13.8%). Our result also found another MDRO, which is *Methicillin-Resistant Staphylococcus aureus* (MRSA) in 3 patients (10.4%) (Table 2). The appearance of Gram-negative bacteria is dominant compared to Gram-positive bacteria (72.42% compared to 27.58%). In the case of Gram-negative, the study showed *Enterobacteriaceae* were seen enormously causing gangrene in DM patients, such as *Klebsiella pneumoniae* (3 patients, 10.4%) and *Proteus mirabilis* (3 patients, 10.4%).

Antibiotic resistance test showed that Gram-negative bacteria were resistant to ampicillin (penicillin type of antibiotic) cefazolin (cephalosporin type of antibiotic) can be seen in Table 3. Through antibiotic resistance test, we found *Pseudomonas putida* showed resistance to almost all antibiotics tested except ampicillin and ampicillin sulbactam, meropenem, and furantoin (Table 3). In the case of *E. coli* ESBL, it is shown that besides cephalosporin resistance (cefazolin, ceftriaxone), it also showed resistance to ampicillin and ciprofloxacin (Table 3). Meanwhile, *E. coli*-ESBL showed susceptibility to carbapenem antibiotics such as ertapenem and meropenem; aminoglycoside such as amikacin; nitrofurantoin, amikacin, and tigecycline. Other Gram-negative bacteria showed resistance to cephalosporins (cefazolin) and penicillin class (ampicillin) can be seen in Table 3. However, it showed susceptibility to carbapenem class (ertapenem and meropenem); aminoglycoside class (amikacin and gentamicin); beta-lactamase inhibitor class alone or in combination with penicillin class (aztreonam, piperacillin-tazobactam); class cephalosporins (ceftazidime, ceftriaxone, cefepime) can be seen in Table 4.

Table 1. Noise value difference test results COVID-19 patients

Patient number	Gender	Age	Bacterial growth
1	Female	47	No
2	Female	47	Yes
3	Male	53	Yes
4	Male	67	No
5	Female	55	Yes
6	Male	65	Yes
7	Male	51	Yes
8	Female	47	Yes
9	Male	73	Yes
10	Male	58	Yes
11	Female	56	Yes
12	Female	58	Yes
13	Male	56	No
14	Male	56	Yes
15	Male	58	No
16	Male	40	Yes
17	Male	53	Yes
18	Female	58	Yes
19	Female	51	Yes
20	Male	48	Yes
21	Female	60	Yes
22	Male	55	Yes
23	Female	55	No
24	Male	40	Yes
25	Male	39	Yes
26	Female	47	Yes
27	Female	51	Yes
28	Female	47	No
29	Male	53	Yes
30	Female	58	Yes
31	Male	50	Yes
32	Female	56	Yes
33	Male	56	No
34	Female	53	Yes
35	Female	51	Yes
36	Male	59	No
37	Female	54	No
38	Female	61	Yes
39	Female	64	No

Table 2. Bacterial profile causing gangrene among DM patient from pus sample

No	Bacteria	Number (Percentage)
Gram-negative bacteria		
1	*Escherichia coli* (ESBL)	4 (13.80)
2	*Klebsiella pneumoniae*	3 (10.35)
3	*Proteus mirabilis*	3 (10.35)
4	*Citrobacter freundii*	2 (6.89)
5	*Citrobacter koseri*	2 (6.89)
6	*Enterobacter cloacae*	2 (6.89)
7	*Enterobacter aerogenes*	1 (3.45)
8	*Morganella morganii*	1 (3.45)
9	*Proteus hauseri*	1 (3.45)
10	*Shigella* sp	1 (3.45)
11	*Pseudomonas putida*	1 (3.45)
Gram-positive bacteria		
12	*Methicillin Resistant Staphylococcus aureus* (MRSA)	3 (10.34)
13	*Enterococcus faecalis*	2 (6.89)
14	*Enterococcus avium*	1 (3.45)
15	*Staphylococcus haemolyticus*	1 (3.45)
16	*Staphylococcus hominis*	1 (3.45)
Total bacterial		29 (100)

The results of the antibiotic resistance test of Gram-positive bacteria showed resistance to tetracycline and ciprofloxacin. Apart from beta-lactam antibiotics, MRSA showed resistance to aminoglycosides class (gentamicin), tetracycline, fluoroquinolone class (moxifloxacin, levofloxacin, ciprofloxacin), lincomycin class (clindamycin) and macrolides class (clindamycin) (Table 5). MRSA showed susceptibility to tigecycline, linezolid, vancomycin, and furantoin. Other Gram-positive bacteria showed susceptibility to linezolid, vancomycin, tigecycline, and ampicillin can be seen in Table 6. Meanwhile, other Gram-positive bacteria showed resistance to tetracycline and susceptibility to linezolid and vancomycin can be seen ini Table 5 and Table 6.

Table 3. Antibiotic resistance results from Gram-Negative bacteria causing gangrene among DM patients

Antibiotics	AMP	SAM	TZP	KZ	CAZ	CRO	FEP	ATM	ERTA	MEM	AK	GN	CIP	F	SXT	TGC
Escherichia coli (ESBL) n=4 (%)	**4 (100)**	2 (50)	1 (25)	**4 (100)**	2 (50)	**4 (100)**	1 (25)	3 (75)	0 (0)	0 (0)	0 (0)	1 (25)	**4 (100)**	0 (0)	3 (75)	0 (0)
Klebsiella pneumoniae n=3 (%)	0 (0)	1 (33)	0 (0)	2 (67)	2 (67)	2 (67)	2 (67)	2 (67)	0 (0)	0 (0)	0 (0)	2 (67)	**3 (100)**	0 (0)	1 (33)	0 (0)
Proteus mirabilis n=3 (%)	2 (67)	1 (33)	1 (33)	**3 (100)**	1 (33)	1 (33)	1 (33)	2 (67)	0 (0)	0 (0)	0 (0)	1 (33)	1 (33)	**3 (100)**	2 (67)	2 (67)
Citrobacter freundii n=2 (%)	**2 (100)**	**2 (100)**	0 (0)	**2 (100)**	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	1 (50)	0 (0)
Citrobacter koseri n=2 (%)	**2 (100)**	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Enterobacter cloaae n=2 (%)	**2 (100)**	**2 (100)**	1 (50)	**2 (100)**	1 (50)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	**2 (100)**	0 (0)	1 (50)	0 (0)
Enterobacter aerogenes n=1(%)	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Proteus hauseri n=1(%)	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Morganella morganii n=1(%)	**1 (100)**	0 (0)	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Shigella sp n=1(%)	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)
Pseudomonas putida n=1(%)	0 (0)	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**

AMP, Ampicillin; SAM, Ampicillin Sulbactam; TZP, Piperacillin Tazobactam; KZ, Cefazolin; CAZ, Ceftazidime; CRO, Ceftriaxone; FEP, Cefepime; ATM, Aztreonam; ERTA, Ertapenem; MEM, Meropenem; AK, Amikacin; GN, Genta-micin; CIP, Ciprofloxacin; F, Nitrofurantoin; SXT, Trimethopprim/sulfamethoxazole; TGC, Tigecycline

Table 4. Antibiotic susceptibility results from Gram-Negative bacteria causing gangrene among DM patient

Antibiotics	AMP	SAM	TZP	KZ	CAZ	CRO	FEP	ATM	ERTA	MEM	AK	GN	CIP	F	SXT	TGC
Escherichia coli (ESBL) n=4 (%)	0 (0)	0 (0)	3 (75)	0 (0)	2 (50)	0 (0)	3 (75)	1 (25)	**4 (100)**	**4 (100)**	**4 (100)**	3 (75)	0 (0)	**4 (100)**	1 (25)	**4 (100)**
Klebsiella pneumoniae n=3 (%)	**3 (100)**	1 (33)	**3 (100)**	0 (0)	1 (33)	1 (33)	1 (33)	1 (33)	**3 (100)**	**3 (100)**	**3 (100)**	1 (33)	0 (0)	0 (0)	2 (67)	0 (0)
Proteus mirabilis n=3 (%)	1 (33)	2 (67)	2 (67)	0 (0)	2 (67)	2 (67)	2 (67)	1 (33)	1 (33)	**3 (100)**	**3 (100)**	2 (67)	2 (67)	0 (0)	1 (33)	0 (0)
Citrobacter freundii n=2 (%)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	1 (50)	**2 (100)**	0 (0)	**2 (100)**	**2 (100)**	**2 (100)**	1 (50)	1 (50)	1 (50)	1 (50)	**2 (100)**
Citrobacter koseri n=2 (%)	0 (0)	1 (50)	**2 (100)**	0 (0)	**2 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	0 (0)	**2 (100)**	1 (50)
Enterobacter cloaae n=2 (%)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	1 (50)	**2 (100)**	1 (50)	1 (50)	**2 (100)**	**2 (100)**	**2 (100)**	0 (0)	0 (0)	1 (50)	1 (50)
Enterobacter aerogenes n=1(%)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	1 (50)	**2 (100)**	1 (50)	1 (50)	**2 (100)**	**2 (100)**	**2 (100)**	0 (0)	0 (0)	1 (50)	1 (50)
Proteus hauseri n=1(%)	0 (0)	0 (0)	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)
Morganella morganii n=1(%)	0 (0)	0 (0)	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)
Shigella sp n=1(%)	0 (0)	0 (0)	**1 (100)**	0 (0)	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonas putida n=1(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

AMP, Ampicillin; SAM, Ampicillin sulbactam; TZP, Piperacillin tazobactam; KZ, Cefazolin; CAZ, Ceftazidime; CRO, Ceftriaxone; FEP, Cefepime; ATM, Aztreonam; ERTA, Ertapenem; MEM, Meropenem; AK, Amikacin; GN, Genta-micin; CIP, Ciprofloxacin; F, Nitrofurantoin; SXT, Trimethopprim/sulfamethoxazole; TGC, Tigecycline

Table 5. Antibiotic resistance results from Gram-Positive bacteria causing gangrene among DM patient

Antibiotics	AMP	OXA	BEN	GN	TGC	SXT	TE	MXF	LEV	CIP	CC	LZD	VA	E	F	Rif
Methicillin Resistant Staphylococcus aureus (MRSA) n=3 (%)	0 (0)	**3 (100)**	0 (0)	**3 (100)**	0 (0)	1 (33)	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	0 (0)	0 (0)	**3 (100)**	0 (0)	2 (67)
Enterococcus faecalis n=2 (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**2 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
Enterococcus avium n=1 (%)	0 (0)	0 (0)	0 (0)	**1 (100)**	0 (0)	0 (0)	**1 (100)**	0 (0)	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	**1 (100)**	0 (0)
Staphylococcus haemolyticus n=1 (%)	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Staphylococcus hominis n=1 (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

AMP, Ampicillin; OXA, Oxacillin; BEN, Benzylpenicillin; GN, Gentamicin; TGC, Tigecycline; SXT, Trimethoprim sulfamethoxazole; TE, Tetracycline; MXF, Moxifloxacin; LEV, Levofloxacin; CIP, Ciprofloxacin; CC, Clindamycin; LZD, Linezolid; VA, Vancomycin; E, Erythromycin; F, Nitrofurantoin; Rif, Rifampicin

Table 6. Antibiotic susceptibility results from Gram-Positive bacteria causing gangrene among DM patients

Antibiotics	AMP	OXA	BEN	GN	TGC	SXT	TE	MXF	LEV	CIP	CC	LZD	VA	E	F	Rif
Methicillin Resistant Staphylococcus aureus (MRSA) n=3 (%)	0 (0)	0 (0)	0 (0)	0 (0)	**3 (100)**	1 (33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**3 (100)**	**3 (100)**	0 (0)	**3 (100)**	1 (33)
Enterococcus faecalis n=2 (%)	**2 (100)**	0 (0)	0 (0)	0 (0)	**2 (100)**	0 (0)	0 (0)	0 (0)	**2 (100)**	**2 (100)**	0 (0)	**2 (100)**	**2 (100)**	0 (0)	1 (50)	0 (0)
Enterococcus avium n=1 (%)	**1 (100)**	0 (0)	0 (0)	0 (0)	**1 (100)**	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**1 (100)**	**1 (100)**	0 (0)	0 (0)
Staphylococcus haemolyticus n=1 (%)	0 (0)	0 (0)	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)	0 (0)	0 (0)	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**
Staphylococcus hominis n=1 (%)	0 (0)	0 (0)	0 (0)	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	**1 (100)**	0 (0)	**1 (100)**	**1 (100)**	0 (0)

AMP, Ampicillin; OXA, Oxacillin; BEN, Benzylpenicillin; GN, Gentamicin; TGC, Tigecycline; SXT, Trimethoprim sulfamethoxazole; TE, Tetracycline; MXF, Moxifloxacin; LEV, Levofloxacin; CIP, Ciprofloxacin; CC, Clindamycin; LZD, Linezolid; VA, Vancomycin; E, Erythromycin; F, Nitrofurantoin; Rif, Rifampicin

DISCUSSION

Demographic information on DM patients reveals that in terms of gender, there is no difference in the probability of DM patients developing gangrene. Meanwhile, in terms of age, DM patients in the group of 45 - 59 years old showed a high probability of developing gangrene. This study is also in accordance with other studies by Tong *et al*. in 2020 that stated *Diabetic Foot Ulcers* (DFU) is common in middle-aged patients. Furthermore, a study conducted in Saudi Arabia showed that >45 years of age can pose a threat to developing DFU (Al-Rubeaan *et al*., 2015; AlSadrah, 2019). This study demonstrated that middle-aged patients with DFUs had worse glycemic control, made worse lifestyle decisions like smoking and drinking, had more severe ulcers, and were more likely to have microangiopathy problems. However, these individuals subsequently recovered more quickly and were at a lower risk of death and significant amputation (Tong *et al*., 2020).

Our result showed that bacteria found in the pus culture dominated by Gram-negative bacteria, with *E. coli* ESBL, MRSA, *Klebsiella pneumoniae*, and *Proteus mirabilis* being the most common bacteria causing gangrene. Gram-negative rods were mostly found in patients with chronic wounds that had already been treated. Beginning in the late 1970s, studies showed that aerobic Gram-positive cocci, particularly *Staphylococcus aureus*, were the most common pathogens in DFIs, frequently as monomicrobial infections. Aerobic DFIs brought on by multidrug-resistant organisms, including extended-spectrum beta-lactamase-producing Gram-negative rods (Varaiya *et al*., 2008) or *Methicillin-Resistant S. aureus* (MRSA), have become a significant issue in recent years. The choice of an active agent against that isolate is undoubtedly necessary in an infection with an antibiotic-resistant bacterium, but therapeutic management should remain unchanged (Uckay *et al*., 2012).

When we added the number of *E. coli* ESBL and MRSA, we found that 24 % of cases were caused by multidrug-resistant bacterial infection. Another factor contributing to the appearance of MDRO in another study by Dubsky *et al*. in 2013 was long-term poor glucose control in DM patients (Dubsky *et al*., 2013) by reducing the therapeutic efficacy of antibiotic therapy, MDRO infection in diabetic foot ulcers makes treatment more difficult and may even result in fatalities or amputations (Agbi *et al*., 2017; Yan *et al*., 2022). The risk factors of drug-resistant negative bacilli in patients should be assessed in developing the initial anti-infective treatment regimen in clinical practice due to the increased resistance rate of *E. coli* ESBL among Gram-negative bacteria. Another study also noted that the appearance of MDRO might be related to the patients with serious illnesses admitted to our hospital as a tertiary care hospital and the more complex history of antibacterial drug use (Yan *et al*., 2022).

Systemic antibiotic therapy is required for patients with poor systemic resistance, which typically coexists with bacteremia or sepsis. As a result, it is crucial to perform a thorough evaluation of each patient with diabetic foot ulcers, keep an eye on the severity of the infection and any changes in the pathogenic bacteria, and consistently administer anti-infective therapy (Tang, 2019). Most *Diabetic Foot Infections* (DFIs) require systemic antibiotic therapy, with empirical selection being the norm at first. Even though numerous antibiotics are available, it is still being determined which is best for treating DFIs. Antibiotic susceptibility: Gram-negative bacteria (wild type and MDRO) showed susceptibility to meropenem class, aminoglycoside class, and beta-lactamase inhibitor antibiotic. Meanwhile, Gram-negative bacteria without MDRO, cephalosporin, and beta-lactamase antibiotics are still susceptible in this study. In the case of Gram-positive bacteria, it showed susceptibility to tigecycline, linezolid, and vancomycin. Other studies suggested that ertapenem with or without vancomycin and tigecycline can treat DFI infection (Olid *et al*., 2015). A study by Du *et al*. in 2022 also showed that Gram-positive bacteria were susceptible to linezolid, vancomycin, and teicoplanin (Du *et al*., 2022).

CONCLUSION

Gangrene or diabetic foot infections occur in the middle age group (45 - 59 years), accounting for 76.9%. Pus culture showed *E. coli* ESBL, MRSA, *Klebsiella pneumoniae*, and *Proteus mirabilis* as the dominant bacteria. Gram-negative bacteria were susceptible to the meropenem class, aminoglycosides class, and tigecycline class of antibiotics. Meanwhile, Gram-positive bacteria were susceptible to tigecycline, linezolid, and vancomycin.

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