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Prevalence and antibiogram of aerobic bacterial isolates from pus samples in a tertiary care hospital of north Kerala, India

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Abstract

Assessment of pathogens diversity and evolving drug-resistant pattern is quite essential in the systematic management of infections. To regulate the pyogenic infection, 1350 (783 males and 567 females) pus samples collected from individuals attending a tertiary care hospital in Northern Kerala. Pathogens isolated from the collected pus samples were identified based on the colony morphology, microscopic examination, and biochemical characteristics. About 84.44% of samples showed significant bacteria. The causative organisms were *Staphylococcus aureus* (28%), *Escherichia coli* (13%), *Pseudomonas aeruginosa* (12%), *Klebsiella pneumonia* (10%), coagulase negative *Staphylococcus* sp. (8%), *Proteus mirabilis* (6%), *Streptococcus* sp. (2%), *Enterococcus faecalis* (2%), *Acenitobactor baumanii* (1%), *Citrobactor koseri* (2%), *Enterococcus faecium* (2%), *Enterococcus* sp. (2%), *Morganella morganii* (1%), *Proteus vulgaris* (2%), and other less prominent bacteria (3%). The drug-resistant pattern of pathogens analyses against 29 contemporary antibiotics. Pathogenic Gram-negative bacteria (GNB) were sensitive to amikacin > imipenem > meropenem > tazobactum > gentamycin > chloramphenicol> ciprofloxacin > levofloxacin and resistant to clindamycin, erythromycin, linezolid, oxacillin, penicillin, and vancomycin. Gram-Positive Bacteria (GPB) were susceptible to linezolid > vancomycin > tetracycline > clindamycin > chloramphenicol > gentamycin > ciprofloxacin, and resistant to amikacin, imipenem, meropenem, and tazobactum. Overall, the study concludes that MDR *S. aureus* was the predominant cause of pyogenic infections, drug resistance pattern of the pathogens in the selected region and raises concerns for the need to analyze signaling mechanism that transforms a susceptible strain into a resistant to develop a suitable treatment strategy.

Keywords: bacterial pathogen; drug resistance; pyogenic infections; *Staphylococcus aureus;* surgical site infection

Introduction

The normal intact skin protects the body against invasive microorganisms by serving as mechanical barrier and retaining microbicidal activity (Perciva *et al.,* 2012). It prevents underlying tissues from becoming colonized and invaded by the potential pathogens. Any injury that causes physical disruption of the skin integrity leads to exposure of subcutaneous tissue, resulting in an infection of the wound by external pathogens

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(Erol *et al.,* 2004). Exposed inflamed tissues in the wound provide a moist environment, and release dead cells (nutritious environment) that are conducive for microbial colonization and proliferation (Oladeinde *et al.,* 2013). Pathogens release cytolytic metabolites like leukocidins that exterminates the macrophages and neutrophils resulting in abscess, suppuration, and discharge of pus. The progression of a wound to a systemic and highly infected state involves a multitude of factors of the host conditions including old age, repeated trauma, blood perfusion, immune suppression, balance between normal flora and exogenous flora, level of antimicrobial peptides from normal skin flora, and co-existing morbidity which impair wound healing and increases the risk of infection (Aitcheson *et al.,* 2021; Crane *et al.,* 2018). Apart from the type, site, size, and depth of the wound and the virulence of the microorganisms facilitate further complications in wound infection (Rodrigues *et al.,* 2019). The order of immune response against pyogenic infections in host are; immune suppressed patients under cancer treatment or undergoing transplantation > diabetic patients (foot ulcers (84%) - poor macrophage phagocytosis)> microbial infections (mycobacteria/ fungi/ bacteria) > mechanism of immune response (Dropulic *et al.,* 2016; Mak *et al.,* 2006; Maoura *et al.,* 2019). For analyzing the key bacterial pathogens, samples from drainage or deep incisions are the suitable samples, and for scheduled retrospective treatment (Hakkarainen *et al.,* 2014).

The etiology of wound infection differs from country to country and from hospital to hospital even within the same region. The key factor governing microbial recurrence is the irregularity in antibiotic treatment that accelerates the emergence of multidrug resistant pathogens which cannot be treated by common antibiotics in use (Rzan Al Battat *et al.,* 2022). Therefore, knowledge of risk factors associated with infections could help to strengthen the efforts towards declining the complications and their recurrence. Standardization of protocol for selection of antibiotic, dosage, and course of treatment are required to reduce morbidity and mortality resulting from pyogenic infections (Alkhafaji Sura *et al.,* 2020).

Even though treatments, especially in life-threatening situations are usually empirical in employing broad-spectrum antibiotics. Especially, control of wound infections has become very challenging due to widespread bacterial resistance to antibiotics such as infection caused by methicillin resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta-Lactamase (ESBL) producers, carbapenem resistant Enterobacterales/pseumonads, and AmpC Beta lactamase producers among Gram negative bacteria (Gupta *et al.,* 2018). Thus, the current work is important to generate findings that would guide the formulation of policies on infection control, empirical antibiotic treatment and control of antibiotic to use.

Materials and Methods

Ethical approval for the present study was issued as per the standard protocols of the committee for the purpose of control and supervision of experiments (CPCSC) on animals and humans, strictly maintained and followed by the Institutional Ethics Committee of MES Medical College Hospital, Kerala (IEC/MES/10/2022; Dated: 20/07/2022). According to the CPCSE guidelines, pus samples were collected from the MES Medical College Hospital, Kerala during January 2021 and December 2021.

Sample collection

All pus samples from patients attending the outpatient department or admitted in wards with infected surgical wounds which were collected following standard procedures and received to the Microbiology lab were used for the study. During the period of study, around 1350 pus samples from wounded sites of both diabetic and non-diabetic patients with complications like venous ulcers, decubitus ulcers, superficial abscesses, and traumatic injuries were collected and included in the study (Figure 1). The patients were belonging to both genders and age group of 01 to 90 years. The criteria used for the sample collection include;

i) The inclusion criteria: The pus sample (pus aspirate and wound swab) from patients attending the outpatient department (OPD) or patients admitted in wards (in patients) with wound infections was collected and stored in the Microbiology clinical laboratory of MES Medical College Hospital, Kerala, India, between January 2021 and December 2021.

ii) Exclusion criteria: Samples collected without following standard guidelines, pus samples collected from patients with more than one complication prescribed by the Association of Indian Medical Society, 2019.

a) b) c)

Figure 1. Types of samples Collected for the study; a. Pus sample collected from diabetic foot ulcer; b. Pus sample collected from bed sore; c. Pus sample collected from acne

Analysis of sample

1) Collected samples were streaked on selective media such as 5% sheep blood agar, Mannitol Salt Agar, and MacConkey agar and incubated at 37 °C for 24h.
2) Direct microscopic examination of Gram's-stained smears of isolates.
3) Additional test included were Coagulase test, Sorbitol fermentation, Arabinose fermentation test, other sugar fermentation test, species specific identification tests, Optochin and Bacitracin sensitivity test, and specific biochemical tests to identify Enterobacteriaceae members.

After 24 hours of incubation, the plates were examined for the growth of the bacteria. Morphological characterization accomplished based on the appearance of the colony on agar plates like colour differences, β or α hemolysis etc. Bacterial characterization of biochemical reactions includes catalase test, oxidase test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test, coagulase test, nitrate reduction test, H_2S production test, Bile Esculin test, and the triple sugar iron agar test.

Antibiotic sensitivity testing

Antimicrobial susceptibility of the isolates was assessed on Muller Hinton Agar plates using Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards institute (CLSI) guidelines. The list of antibiotics tested include amikacin (Amk-30 µg), ampicillin (Amp-10 µg), bacitracin (Bac-10 µg), cefipime (Cef-30 µg), ceftazidime (Cez-30 µg), cefoxitin (Cex-30 µg), cefotaxim (Cet-30 µg), ceftriaxone (Ceo-30 µg), cefuroxime (Ceu-30 µg), chloramphenicol (Chl-30 µg), ciprofloxacin (Cip-5 µg), clindamycin (Cli-2 µg), colistin (Col-10 µg), erythromycin (Ery-15 µg), gentamycin (Gen-10 µg), gentamycin high (GeH-120 µg), imipenim (Imp-10 µg), levofloxacin (Lev-5 µg), linuzolid (Lin-30 µg), meropenam (Mer-10 µg), oxacillin (Oxa - 1 µg), penicillin (Pen-10 µg), piperacillin (Pip-10 µg), tazobactum (Taz -10 µg), polimixin B (Pol-300 µg), tetracyclin (Tet-30 µg), clotrimazole (Clo-1.25 µg), teicoplanin (Tei-30 µg), and vancomycin (Van-30 µg).

Results

Sample collection

According to the inclusion criteria, around 1350 samples were collected aseptically from the Microbiology laboratory of MED Medical College Hospital, Perinthalmanna, Kerala, India. From the collected 1350 samples, 1140 samples showed significant bacterial growth, indicating a microbial infection in the patients. It was confirmed that 84.44% of the wounded sites were further aggravated by microbial infections from external sources (Table 1).

Table 1. Gender wise distribution of pathogens

Samples	Number of samples collected	Culture positive	Percentage of positive cases
Total	1350	1140	84.44
Male	783	673	85.95
Female	567	467	82.36

The complications associated with wound infections are determined by the patient's age, gender, and immune strength. Among the 1140 positive samples, the majority were obtained from the 51 to 60 years old age group (270, 24%). Even though predominantly male patients attending in this age group (140, 13%) were affected during this age group, whereas in female patients, the largest number (101, 8.8%) of female samples belong to the age group 61 to 70 years. The age-wise distribution of samples is given in Figure 2. Samples from surgical wounds, open wounds with pus, diabetic foot ulcers, and pus from burn wounds were collected. Among them, the patient's age is the major factor influencing the microbial infection at the wounded site, as the immune system is in a deprived condition.

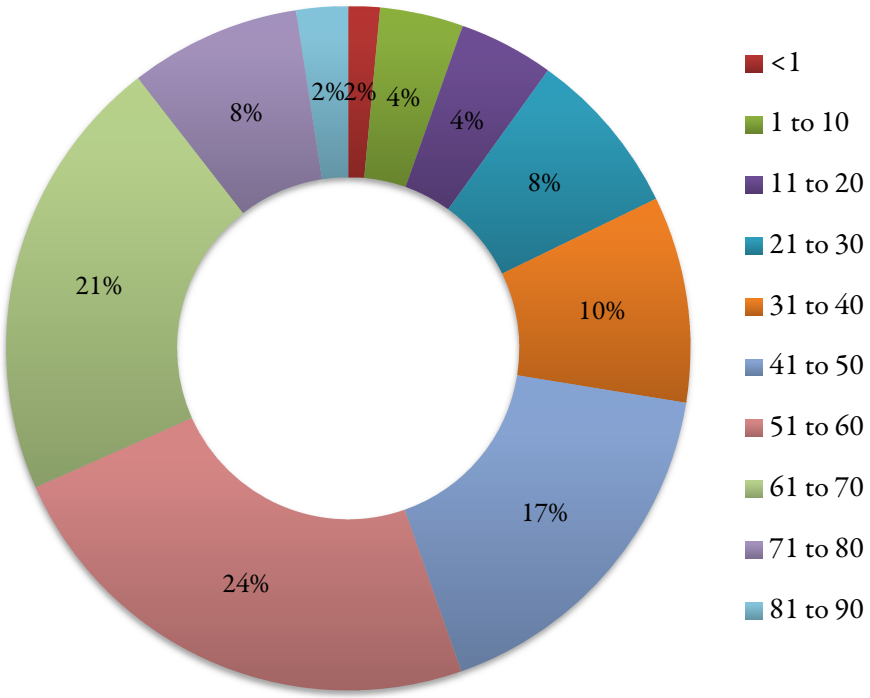
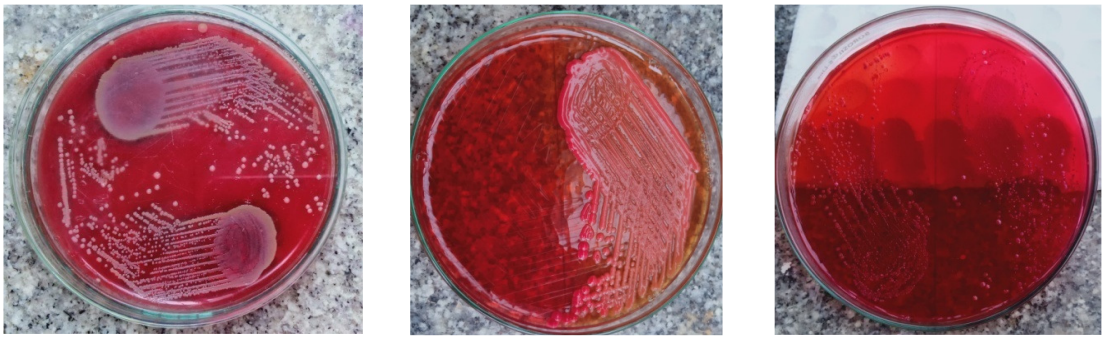


Figure 2. Age-wise (in years) wound infection rate in collected samples

Isolation of bacteria from pus samples

The samples streaked on selective agar plates were observed for growth, colour of the colony, and colony morphology after 24 hours of incubation (Figure 3). Gram positive bacteria (GPB) and Gram-negative bacteria (GNB) were isolated from the samples; most of the GPB belonged to the cocci, and the GNB were rod shaped. Gram staining indicated 512 (44.91%) Gram positive bacteria (GPB) and 628 (58.09%) Gram negative bacteria (GNB). As the skin's normal flora is *Staphylococcus* sp., most of the pus isolates were belonging to the opportunistic GPB, *Staphylococcus* sp. Growth in culture media, cultural characteristics, and the colour of the colony were recorded. Typical colony morphology with colour change of the colonies on selective media indicated the immediate identification of a specific isolate, and the isolate, which did not respond to common identification tests and in ambiguous result, was further subjected to specific confirmatory tests such as growth at 44 °C, niacin sensitivity, arginine utilization, etc., to identify the organism.



a) b) c)

Figure 3. Bacterial growth on positive culture plates; a) *S. aureas* growth on blood agar; b) *Klebsiella* sp., growth on MacConkey agar; c) *E. coli* growth on MacConkey agar

Biochemical tests for identification of pathogens

Biochemical tests were performed, and the results are recorded in Table 2. Routine microscopic examination in combination with colony morphology and certain biochemical tests confirmed the commonly occurring pathogens. The predominant bacterial isolates were *Staphylococcus aureus* in 317 samples (28%), *Staphylococcus* sp. Coagulase negative in 95 samples (8%), *Streptococcus* sp., in 20 samples(2%), *Escherichia coli* in 149 samples(13%), *Klebsiella pneumonia* in 118 samples (10%), *Proteus mirabilis* in 70 samples (6%), *Enterococcus faecalis* in 26 samples (2%), *Pseudomonas aeruginosa* in 139 samples (12%), *Acenitobactor baumanii* in 16 samples (1%), *Citrobactor koseri* in 21 samples (2%), *Enterococcus faecium* in 21 samples (2%), *Enterococcus* sp., in 24 samples (2%), *Morganella morganii* in 17 samples (1%), *Proteus vulgaris* in 21 samples (2%), and 36 samples of bacteria (3%) belonging to other less prominent bacterial isolates. *Staphylococcus aureus* (28%) was the predominant bacterial flora isolated from the pus samples received in the Microbiology Clinical Laboratory, MES Medical College Hospital, in 2021. This was followed by *E. coli* with 13% positivity. Therefore, the *S. aureus* infection was considered primary among the patients (Figure 4).

Table 2. Microscopic, colony morphology and biochemical characteristics used to identify the pathogens

Enterococcus faecium	*Enterococcus faecalis*	*Escherichia coli*	*Enterobacter aerogens*	*Citrobacter* sp.	*Citrobacter koseri*	*Burkholdaria* sp.	*Acenitobactor* sp.,	*Acenitobacter baumanii*	**Organism / Tests**
Oval shaped gram-positive cocci in pairs	Oval shaped gram-positive cocci in pairs	GNB	GNB	GNB	GNB	Gram negative Bacilli in bipolar safety pin appearance	Gram Negative cocco-bacilli in pairs. thump print appearance	Gram negative cocco-bacilli in pairs. Thump print appearance	**Gram stain**
		motile	motile	motile	motile	non motile	non motile	non motile	**Wet mount**
		Lactose fermenting pinkish colonies						Pinkish colony	**Culture**
		+	I	+	+				**Indole**
		+		+					**MR**
		I		'					**VP**
		I	+	+					**Citrate**
		I	I	I					**Urease**
						+	I		**Oxidase**
									Catalase
									Coagulase
		A/A	A/A with GAS	A/A	K/A		-	A/A	**TSI**
		+				+			**Lactose**
							I		**Nitrate**
		I		+	I				**H2S**
									Bile Esculin
I	+								**Sorbitol**
+	I								**Arabinose**
						PB Colistin resistant		Growth at 44 C	**Other tests**

Proteus vulgaris	*Pseudomonas* sp.	*Proteus mirabilis*	*Burkholdariapseudo mallei*	*Burkholdaria cepacia*	*Pseudomonas aeroginosa*	*Morganella morganii*	*Klebsiella pneumonia*	*Klebsiella oxytoca*	*Enterococcus* sp.	*Enterobactor* sp.
GNB	GNB	GNB	GNB	GNB	GNB	GNB	GNB	GNB	Oval shaped gram-positive cocci in pairs	GNB
Motile	Motile	Motile	Non-motile	Motile		Motile	Non motile	Non-motile		Motile
	Earthy smell colonies, non-lactose fermenting	fishy order	no growth in MacConkey agar		non lactose fermenting, Greenish pigmented colonies		pinkish mucoid colonies	pinkish mucoid colonies		
+		I			I	+	I	+		I
		+			I	+	I			I
		I			I	I	+			+
						I	+	+		+
+		+				+	+	+		
				+	+					
					+					
				A/A	K/NR	K/A				
					I		+	+		
					+					
		+				I	+			I
									+	
				Niacin and Arginine positive					Optochin & Bacitracin resistant	

Streptococcus sp.	*Streptococcus pyogenes*	*Serratia marcescenes*	*Serratia* sp.	*Staphylococcus*, Coagulase negative	*Staphylococcus aureus*
gram positive cocci in chains	gram positive cocci in chains	GNB	GNB	Gram positive cocci in clusters	Gram positive cocci in clusters
		motile	motile		
α or β hemolytic colonies on BA	β - hemolytic colonies on BA	pink or red pigmented colonies	pink or red pigmented colonies		β hemolytic colonies on BA, pinkish colonies on MC
		I	I		I
		I	I		+
		+	+		+
		+	+		
		+			+
		I			
'		+			+
				I	+
			K/A		
		+	+		+
			+		
				yellow colonies in MSA	yellow colonies in MSA

Key words: "+" = positive test result; "I" = Negative test result; "A" = acid production; "K" = alkaline condition; "G" = Gas production; "NR"= non-reactive, "GNB" = Gram negative bacteria; "GPB" = Gram positive bacteria; MSA = Mannitol salt agar, BA= Blood Agar, MC = Mec-Conkey Agar

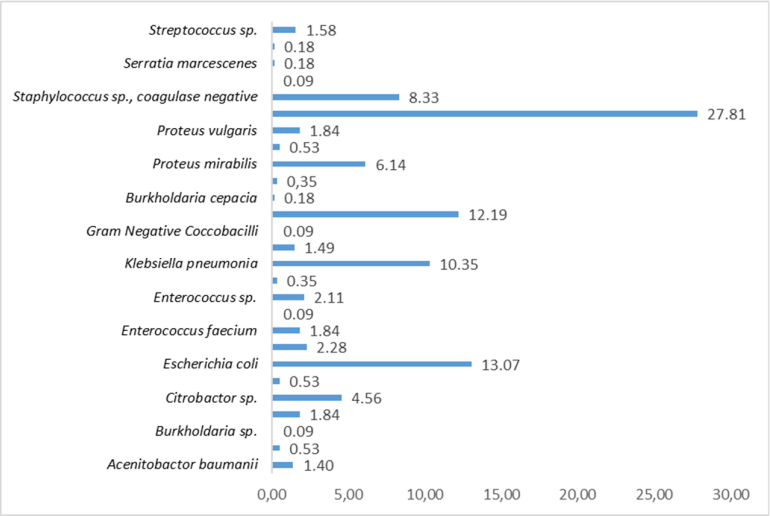


Figure 4. Bacterial population isolated from wound samples

Isolation of bacteria from diabetic foot samples

Of the 1350 wound samples, a total of 597 diabetic foot ulcer samples were streaked on the agar plates and observed for growth. A total of 581 bacterial growth positives were observed. These isolates were examined morphologically and biochemically to identify the pathogen. The pus samples were predominantly males (60.4%) compared to females (39.6%) in cases of diabetic foot ulcers. Aerobic Gram-negative organisms were more frequently (63.5%) isolated compared to Gram-positive organisms (36.5%) in our study even, though *Staphylococcus aureus* was the most frequently isolated Gram-positive bacteria from diabetic foot infections (Sannathimmappa *et al*., 2021) in other studies. With regard to age-wise distribution among culture-positive samples, patients between 51 and 60 years old contributed 35.8% of culture-positive samples (Figure 5).

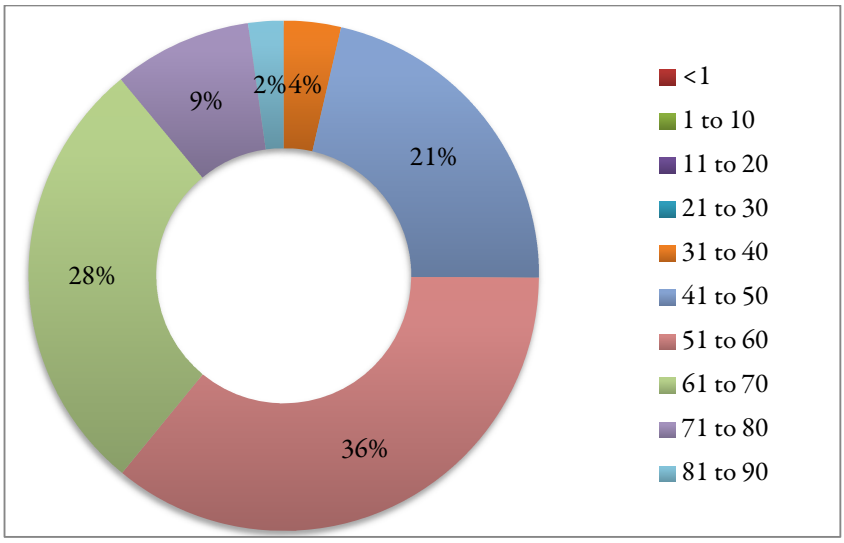


Figure 5. Age-wise (in years) distribution of clinical pathogens in diabetic foot ulcers patients

Antibiotic sensitivity pattern of pathogen

Each isolate was examined for antibiotic sensitivity against common antibiotics. Among the 317 clinical isolates of *S. aureus,* 136 (43%) were methicillin-resistant and among the 149 isolates of *E. coli,* about 36

(24.1%) was belong to the extended spectrum of β-lactamase resistant pathogens. These results were concerning (Figure 4 and Table 3). The isolated Gram-negative bacteria (GNB) were sensitive to antibiotics such as amikacin > imipenem > meropenem> tazobactum > gentamycin > chloramphenicol > ciprofloxacin > levofloxacin and were resistant to clindamycin, erythromycin, linezolid, oxacillin, penicillin, and vancomycin. In Gram Positive Bacteria (GPB), susceptibility to linezolid > vancomycin > tetracycline > clindamycin > chloramphenicol > gentamycin > ciprofloxacin, and resistance to amikacin, imipenem, meropenem, and tazobactum were recorded (Figure 6).

Table 3. Antibiotic resistant pattern of isolates from pus samples of wound infections

Organisms	*Amikacin*	*Ampicillin*	*Bacitracin*	*Ceftpime*	*Ceftazidime*	*Cefoxitin*	*Cefoaxim*	*Ceftriaxone*	*Cefuroxime*	*Chloramphenicol*	*Ciprofloxacin*	*Clindamycin*	*Colistin*	*Erythromycin*	*Gentamycin*	*Gentamycin High*	*Imipenim*	*Levofloxacin*	*Linuzolid*	*Meropenam*	*Oxacillin*	*Penicillin*	*Piperacillin*	*Tazobactum*	*PolimixinB*	*Tetracyclin*	*Clotrimazole*	*Teicoplanin*	*Vancomycin*
Acenitobactor baumanii	2										1		13		1		2	1		2				2		4	1		
***Acenitobactor* sp.**	1												5				2			1						5	3		
Burkholdaria sp.	0			1	1						1						1	1								1	1		
Citrobactor koseri	20			17	13	6	6	13	6	16	16		1		17		21	16		20				16	1	12	3		
***Citrobactor* sp.**	41			37	33	3	13	32	22	40	39		7		38		49	38		43				41	8	37	16		
Enterococcus aerogens	6			4	4	1	1	3		3	5		1		5		6	5		6				4	1	3	1		
Escherichia coli	129	8		58	43	34	23	46	30	106	62		12		108		123	62		122				117	11	61	14		
Enterococcus faecalis	0	21									9			13		21		9	25			22				4			26
Enterococcus faecium	0	8									4			1		9		3	20			8			3			19	
***Enterobactor* sp.**	1			1	1			1	1	1	1				1		1	1		1				1		1	1		
***Enterococcus* sp.**	0	22									5			8		15		8	24			21			6	1		23	
Klebsiella oxytoca	3			3	3	1	1	3	1	3	3		1		3		3	3		3	2				1	3	2			
Klebsiella pneumonia	88			61	50	23	28	50	40	54	71		12		75		97	72		94						58	19			
Morganella morganii	17			15	14	2	9	13	1	10	12				13		17	12		17				17		4	4			
Gram Negative coccobacilli	1									1					1		1			1										
Pseudomonas aeroginosa	113			112	106		2				94		16		103		123	88		120			17	117	15					
Burkholdaria cepacia	0			1	1		1	1			1						2	1		2				2			1			
Burkholdaria pseudomallei	2			4	4			4	1		3				1		4	3		4				4		3	2			
Proteus mirabilis	66	30		65	63	25	31	63	49	38	56				61		68	59		69				67			19			
***Pseudomonas* sp.**	5			4	3						4		3		4		5	4		5				4	3					
Proteus vulgaris	20			20	16	12	9	16	4	8	19				20		20	19		20				21						
Staph. aureus		2				67		16		247	154	217			245			22	302		132	34				301	2			
Staph. Coagulase negative						12		2		62	52	49		24	42			10	94		27	22				64	22	18	95	
***Serratia* sp.**	1			1	1			1		1	1				1		1	1						1			1			
Serratia marcescenes	2			2	2	1	1	2		2	2				2		2	2		2				2		1	1			
Streptococcus pyogenes		2	1					1		1		2		1					2							1			2	
***Streptococcus* sp.**		16	4							8		18		14					18								6		18	
Total	**518**	**109**	**5**	**406**	**358**	**187**	**126**	**268**	**155**	**601**	**615**	**286**	**71**	**170**	**741**	**45**	**548**	**452**	**485**	**534**	**159**	**107**	**17**	**504**	**55**	**580**	**225**	**66**	**486**

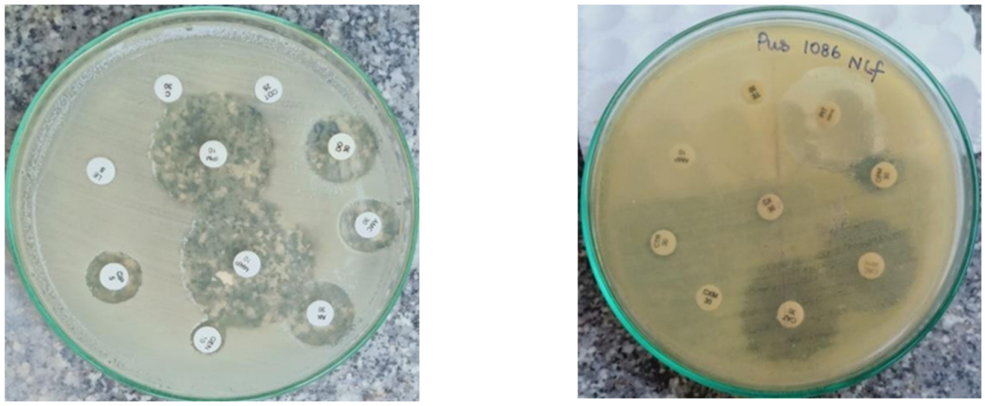


Figure 6. Antibiotic sensitivity pattern of isolates from infected wound sample (bacterial isolate collected from pus sample no. 758 and 1086)

Discussion

The distribution of aerobic bacteria in pus samples from infected individuals between January 2021 and December 2021 was analyzed**.** Out of 1350 samples, 1140 (84.44%) samples showed significant microbial infection in the patients. Surgical and non-surgical wound infections are caused by the normal flora, like *Streptococcus* sp., *Propionibacter* sp., and *Staphylococcus* sp., from the near-by area introduced to the infected area through cross-contamination. In 2018, the Centre for Disease control (CDC, WHO) reported that 20% of the women who underwent caesarean sections contracted wound infections, and 11% of the males developed pyogenic infections immediately after surgery from drainage from nearby sites or the use of immune suppressive medications. In the current study, around 80% of the samples showed microbial infections in the wound, either from severely wounded patients attending the clinic or due to poor hygienic practices or poor knowledge of the patient's medical practices. These are the key governing factors responsible for health care-associated infections (Mohan *et al.,* 2021).

The distribution of GPB and GNB was 512 (45%) and 628 (55%), respectively. GPB and GNB cause pyogenic infections in individuals. The bacterial incidence was highly based on the severity of the disease, the immunity of the patient, and also the effect of predisposing factors (Mohan *et al.,* 2021). GNB outnumbered GPB in a general hospital in the nearby states of North Kerala, like in Andhra Pradesh, India (Sumanth *et al.,* 2020), but in this study, GPB dominated GNB. Among the Gram-positive cocci, *S. aureus* was the most predominant organism (Roopshree *et al.,* 2021) in Andra Pradesh, Bangalore, and Karnataka respectively. According to them, the major isolate from pyogenic infection was MRSA (43%). In the present study, among the 317 *S. aureus* positive cases 136 (42.90%) were MRSA. This may be because the three are neighboring states and may have a common protocol in the use of antibiotics. Moreover, maintenance of wound hygiene is important, and correct empirical monitoring is required to control the evolution of multidrug-resistant strains among individuals.

S. aureus produces various toxins; among them, the δ-toxin induces the granulation of mast cells, which promotes both innate and adaptive type 2 immune responses; another α-toxin also induces IL-1β production from monocytes, which may consequently promote a T_H17 response; or from $CD4^+$ T cells, making the cytokine IL-17. By contrast, when exposed to S. aureus-derived cell wall component lipoteichoic acid, T cells neither proliferated nor produced cytokines, indicating that *S. aureus* products activate the immune system and also temporarily paralyses it. In addition to targeting immune cells, *S. aureus* has also been shown to trigger adipocytes to rapidly proliferate and to produce increased levels of the antimicrobial peptide cathelicidin as a

host defence mechanism (Byrd *et al.,* 2018). These are the ways in which the bacteria outnumber others and proliferate at the infected site, thereby predominately causing the pyogenic infections.

It has also been reported that apart from *Staphylococcus aureus* many GNB are involved in causing pyogenic infections; these include *E. coli* (Sumanth *et al.,* 2020; Roopa shree *et al.,* 2021), *Pseudomonas aeroginosa*, *Klebsiella* sp., (Divya shanthi *et al.,* 2015), *Citrobactor* sp., *Proteus* sp., etc. Pyogenic infections are common among those with poor hygiene. GNB are susceptible to amikacin and meropenem (Roopashree *et al.,* 2021; Divya shanthi *et al.,* 2015), and in GPC, the pattern shows susceptibility to linezolid and vancomycin. In this study, the susceptibility of GNB to amikacin and meropenem was 100%, and susceptibility of GPC to linezolid and vancomycin was 94.7%. Among the Gram-negative isolates (n = 81, 54.73%), 60 (74.07%) were multidrug resistant, with the majority being susceptible to imipenem, meropenem and amikacin (Roopashree *et al.,* 2021). Among the 36 ESBL strains, 32 (88.88%) were susceptible to imipenem, meropenem, and amikacin. The frequency of isolation was predominant among males (65%) (Shivra *et al.,* 2020). Aerobic Gram-negative rods were predominantly (56.5%) isolated compared to Gram-positive organisms (43.5%), especially from male volunteers (60.4%) rather than female volunteers (39.6%). *Staphylococcus aureus* was the most frequently isolated Gram-positive bacteria from pyogenic infections (Bhumbla *et al*., 2019; Roopashree *et al.,* 2021). Most of the patients were in the 41 - 60 age group (Byrd *et al.,* 2018; Divyashanthi *et al.,* 2015; Shivra Batra *et al.,* 2020). Possible reported reasons for the evolution of 50% of the drug resistant pathogens were inappropriate use of antibiotics by the patients, high risk of drug-resistant pathogens (especially MRSA) by the health care workers among the patients, and/or public illiteracy on the antibiotic schedule (Mulay *et al.,* 2022). In this study, the most prominent age group was 51 to 60, followed by 61 to 70 and 41 to 50 years. With regard to diabetic foot infections, aerobic Gram-negative rods were predominantly isolated compared to Gram-positive organisms. Among 581 culture-positive samples, 369 (63.5%) were GNB. The isolation frequency was predominant in males. In this study, male volunteers isolated 60.4% of the total culture positive diabetic foot infections causing pathogens. The age group of the patients was 65±11 (Mohan *et al.,* 2021). Regarding the age-wise distribution, 51-60 years olds contributed 35.8%, followed by the 61- to 70-year-old age group. Diabetic foot infections are most prominent due to their diabetic neuropathic effects (El Boullant *et al.,* 2022). This is a retrospective study in which we could not investigate the significant determinants such as the source of infection, the duration of hospital stays, and clinical outcome. In addition, the study was based on the characterization of bacterial isolates based on phenotypic, conventional, and automated methods only.

Conclusions

Disturbances caused by changes in the normal flora and the contamination of wounds by pathogenic bacteria may lead to pyogenic infections; this may cause pathogenic microflora to multiply and further lead to diseases. The normal flora of every individual is not the same and depends on wound hygiene. Gram positive bacteria were the predominant causative organisms of pyogenic infection. The predominant bacterial isolates were *S. aureus* (28%), followed by *E. coli* in 149 (13%), *P. aeruginosa* (12%), and *Klebsiella pneumonia* (10%).

The antibiotic sensitivity pattern with culture positive samples was evaluated. The prominent antibiotic susceptibilities for GNB are imipenem (86.1%), followed by amikacin (81.5%), meropenem (83.92%), tazobactum (79.45%), chloramphenicol (44.4%), ciprofloxacin (61.30%), gentamycin (71.33%), levofloxacin (60.82%), and resistance to clindamycin, erythromycin, linezolid, oxacillin, penicillin, and vancomycin. Among GPC, the susceptibility pattern is vancomycin (95.11%), followed by linezolid (94.92%), tetracycline (75.58%), clindamycin (56%), chloramphenicol (63.08%), ciprofloxacin (45.11%), gentamycin (57.42%), and resistance to amikacin, imipenem, meropenem, tazobactum, etc. This study shows chloramphenicol, ciprofloxacin, gentamycin, tetracycline, and clotrimazole are susceptible to some strains of both GPC and GNB. Around half of the isolated *Staph. aureus* (43%) evolved as MDR strains, especially vancomycin, oxacyllin, chloramphenicol,

and ciprofloxacin; in GNB, one fourth of the isolated *E. coli* were MDR and commonly resistant to amikacin, imipenim, meropenem, and tazobactum. So, the treatment of pyogenic infections has become very challenging due to widespread bacterial resistance to antibiotics. The study is important to generate findings that would guide the formulation of policies on infection control, empirical antibiotic treatment, and control of antibiotic use.

Routine surveillance for pathogens and their susceptibility to antibiotics is of paramount importance, not only to reinforce strategies for successful pathogenic bacterial pyogenic infection control and management (Natasya *et al.,* 2021). The study helps to provide guidance for using the appropriate antibiotic regimen for effective treatment and to prevent discriminatory usage of antibiotics.

Authors' Contributions

RK: Collection of data, collection of samples, processing of sample, carrying out tests, recording of results and manuscript writing; **PSK:** Correction of the data, results analysis and interpretation, editing of manuscript and correspondence works; **SMA:** Standardization and regulation of methodology.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Ethical approval for the present study was issued as per the standard protocols of the committee for the purpose of control and supervision of experiments (CPCSC) on humans, strictly maintained and followed by the Institutional Ethics Committee of MES Medical College Hospital, Kerala (IEC/MES/10/2022; Dated: 20/07/2022).

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aitcheson SM, Frentiu FD, Hurn SE, Edwards K, Murray RZ (2021). Skin wound healing: normal macrophage function and macrophage dysfunction in diabetic wounds. Molecules 26(16):4917. *https://doi.org/10.3390/molecules26164917*

Alkhafaji Sura L, Kashamar Abbass M, Alkhafaji Ibrahim (2020). Chemical composition and antimicrobial activity of different solvent extracts of *Carthamus tinctorius* flowers. Research Journal of Pharmacy and Technology. 13(12):6055-6060. *https://doi.org/10.5958/0974-360X.2020.01055.0*

Bhumbla U, Safaa Muneer Ahmed, Dinesh Raj Mathur, AS Dalal (2019). A study of wound infections and its antibiogram in surgical intensive care unit of a tertiary care hospital. Tropical Journal of Pathology and Microbiology 5(7):473-478. *https://doi.org/10.17511/jopm.2019.i07.10*

Byrd, A, Belkaid Y, Segre J (2018). The human skin microbiome. Nature Reviews in Microbiology 16:143-155. *https://doi.org/10.1038/nrmicro.2017.157*

Crane MJ, XuY, Henry WL Jr Gillis SP, Albina JE, Jamieson AM (2018). Pulmonary influenza A virus infection leads to suppression of the innate immune response to dermal injury. PLoS Pathogen 14(8):e1007212. *https://doi.org/10.1371/journal.ppat.1007212*

Divyashanthi CM, Adithiyakumar S, Bharathi N (2015). Study of prevalence and antimicrobial susceptibility pattern of bacterial isolates in a tertiary care hospital. International Journal of Pharmacy and Pharmaceutical Sciences 7(1):185-190.

Dropulic LK, Lederman HM (2016). Overview of infections in the immunocompromised host. Microbiology Spectrum 4(4):10.1128. *https://doi.org/10.1128/microbiolspec.dmih2-0026-2016*

Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M (2004). Changes of microbial flora and wound colonization in burned patients**.** Burns 30:357-361. *https://doi.org/10.1016/j.burns.2003.12.013*

Fatima A, Gohar H, Dawood K, Siddiqui HZ, Sajjad M, Naseem S (2022). Bacteriological profile and antimicrobial susceptibility pattern of pus isolates from tertiary care hospital. Journal of Liaquat University of Medical & Health Sciences 21(03):190-195.

Gandra S, Tseng KK, Arora A, Bhowmik B, Robinson ML, Panigrahi B, ... Klein EY (2019). The mortality burden of multidrug-resistant pathogens in India: a retrospective, observational study. Clinical Infectious Diseases 69(4):563-570. *https://doi.org/10.1093/cid/ciy955*

Gupta V, Garg R, Kaur M, Garg S, Attri AK, Chander J (2015). Prevalent resistance mechanisms in isolates from patients with burn wounds. Indian Journal of Burns 23:60-64. *http://dx.doi.org/10.4103/0971-653X.171659*

Hakkarainen TW, Kopari NM, Pham TN, Evans HL (2014). Necrotizing soft tissue infections: review and current concepts in treatment, systems of care, and outcomes. Current Problems in Surgery 51(8):344-362. *https://doi.org/10.1067/j.cpsurg.2014.06.001*

Haque M, Sartelli M, McKimm J, Abu Bakar M (2018). Health care-associated infections - an overview. Infection and Drug Resistance 11:2321-2333. *https://doi.org/10.2147/idr.s177247*

Kumar GS, Sreedevi S, Krishna JB (2020). One year study of aerobic bacterial profile and antimicrobial susceptibility pattern of pus samples. Indian Journal of Microbiology Research 7(1):1-5. *https://doi.org/10.18231/j.ijmr.2020.001*

Mak TW, and Saunders ME (2006). Immunity to pathogens. The Immune Response 641-694. *https://doi.org/10.1016/B978-012088451-3.50024-7*

Moura J, Madureira P, Leal EC, Fonseca AC, Carvalho E (2019). Immune aging in diabetes and it simplications in wound healing. Clinical Immunol**ogy** 200:43-54. *https://doi.org/10.1016/j.clim.2019.02.002*

Mulay MV, Kulkarni SS, Mulay VV (2022). Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* isolates from anterior nares of healthcare workers of a tertiary care hospital. JKIMSU 11(2):1-8

Oladeinde B, Omoregie R, Olley M, Anunibe JA, Onifade AA (2013). 5 - year surveillance of wound infections at a rural tertiary hospital in Nigeria. African Health Sciences 13(2):351-356. *http://dx.doi.org/10.4314/ahs.v13i2.22*

Perciva SL, Emanuel C, Cutting KF, Williams DW (2019). Microbiology of the skin and the role of biofilms in infection. International Wound Journal 9(1):14-32. *https://doi.org/10.1111/j.1742-481x.2011.00836.x*

Rodrigues M, Kosaric N, Bonham CA, Gurtner GC (2019). Wound healing: a cellular perspective. Physiology Reviews 99:665-706. *https://doi.org/10.1152/physrev.00067.2017*

Roopashree S, Prathab AG, Sandeep T (2021). Bacteriological profile and antibiotic susceptibility patterns of wound infections in a tertiary care hospital in South India; Indian Journal of Microbiology Research 8(1):76-85. *http://dx.doi.org/10.18231/j.ijmr.2021.016*

Rzan A Al-Battat, Basman Medhat Fadheel, Shatha Al-Bayati (2022). Rifampicin versus doxycycline in prevention of recurrent boils (A comparative therapeutic study). Research Journal of Pharmacy and Technology. 15(7):3041-3046. *https://doi.org/10.52711/0974-360X.2022.00508*

Sannathimmappa MB, Nambiar V, Aravindakshan R, Al Khabori MSJ, Al-Flaiti AHS, Al-Azri KNM, Al-Reesi AKS, Al Kiyumi ARM (2021). Diabetic foot infections: Profile and antibiotic susceptibility patterns of bacterial isolates in a tertiary care hospital of Oman. Journal of Education and Health Promotion 10:254. *https://doi.org/10.4103/jehp.jehp_1552_20*

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