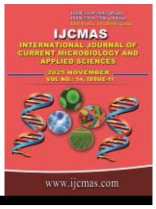
Int.J.Curr.Microbiol.App.Sci (2025) *14*(11): 337-345

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 14 Number 11 (2025)
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2025.1411.035

Organism Profile and Antibiotic Susceptibility Pattern Analysis of Diabetic Foot Patients of a Rural Tertiary Care Hospital in South India

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ABSTRACT

Diabetic foot infections (DFIs) are serious public health issues. The accurate knowledge of the organisms and their susceptibility pattern would improve rational use of antibiotics and prevent resistance. The aim was to investigate the organism profile and antimicrobial susceptibility pattern of microbes in DFIs. A retrospective, cross sectional study was conducted for a period of 1 year at the Department of General Surgery, Malabar Medical College Hospital and research centre, Kerala, India. During this period, 106 patients having DFIs admitted in the general surgery wards were tracked from the hospital data management system Samples of pus, swab and whole blood from the patients were tested for organisms and their antibiotic susceptibity. Consecutive sampling technique was used. Antimicrobial susceptibility test was performed by disc diffusion techniques according to Clinical and Laboratory Standards Institute guidelines. Of the 106 specimens of the diabetic foot lesions, culture showed polymicrobial growth in 34.9% (37/106), monomicrobial growth in 50.9% (54/106), and no growth in 14.2% (15/106). Microbiological evaluation of diabetic foot ulcer infections showed that the prevalence of Gram-negative organisms (79.10%, 106/134) and Gram-positive organisms (20.89%, 28/134). *Klebsiella species* was the most frequent pathogens (19.40% [26/134]). *Staph aureus* (13 [46.42%]) isolates were the bacterial species most commonly isolated among the Gram-positive bacteria. *Staph aureus* isolates were 100% sensitive to rifampicin and daptomycin. *Staph haemolyticus* showed 100% sensitivity to tigecycline, vancomycin, and linezolid. *E. faecalis* isolates were 100% susceptible to linezolid and daptomycin. The current study shows that the prevalence of Gram-negative organisms was greater than that of Gram-positive organisms. DFU was shown to be more prevalent in men than in women. In our study, a greater percentage of monomicrobial infections was found. The DFU was significantly higher in patients with uncontrolled diabetes. *Klebsiella species* were the most common microorganisms found in the diabetic foot ulcer patients. The study emphasizes the need for preventing the DFI by stricter diabetic control measures and rational use of antibiotics with correlation to organisms and their susceptibility pattern.

Keywords

Antibiotic resistance, Antimicrobial susceptibility, Diabetic foot infection, Diabetic foot ulcer, Multidrug-resistant organism

Article Info

Received:
20 September 2025
Accepted:
31 October 2025
Available Online:
10 November 2025

Introduction

Diabetes mellitus represents a set of autoimmune, metabolic and genetic disorders 1. There are three major types of diabetes: (1) Type 1, in which the pancreas does not produce insulin; (b) type 2 in which the body cells are resistant to the action of insulin that is being produced and over time the production of insulin progressively decreases; and (c) gestational diabetes which occurs in pregnancy and can cause some complications during the pregnancy, Uncontrolled diabetes leads to complications in many organs. Diabetes causes disability and shortens lives. The first WHO Global Report on Diabetes was launched on World Health Day 7th April 2016 The number of people with diabetes has increased from 180 million in 1980-422 million in 2014.2 Diabetic foot ulcers (DFU) are a common consequence of peripheral artery disease (PAD) and diabetic neuropathy, and they are often exacerbated by infection. 3 Increased morbidity and mortality as well as a markedly lower quality of life are caused by foot ulcers and amputations. Diabetes mellitus is increasing by epidemic proportions resulting in end-organ damage due to many years of hyperglycemia presenting a major burden of health care.4 It is estimated that approximately 15% of the more than 150 million people with diabetes world-wide will at some stage develop diabetic foot ulceration. 5 DFI represents a major medical, social, and economic problem all over the world. Although the initial care of these infections consists of empirical antimicrobial therapy, which is frequently based on susceptibility data, proper management demands the appropriate selection of antibiotics based on culture and antimicrobial susceptibility results. Therefore, this study was designed to assess the sensitivity pattern and prevalence of microorganisms in infected diabetic foot at a rural tertiary care hospital in South India.

Materials and Methods

Study design and setting

A retrospective, descriptive study of the all the inpatients under the General Surgery department, between the period of 1st January 23 to December 31st 2023.During the study period obeying inclusion and exclusion criteria was grouped by using consecutive sampling technique. The organism and culture sensitivity reports were collected from the VITEK software of the Microbiology department.

Sample size

A total of 106 diabetic patients were included in the study, the sample size was calculated using the sample size determination Fischer"s formula.

Inclusion Criteria

Medical records of all inpatients in the surgery ward who was treated with Diabetic Foot Infection within the study period.

Exclusion Criteria

Any other ulcer patients (arterial ulcers, venous stasis ulcers) who are not suffering from Diabetic Foot Infection.

Data collection

A retrospective analysis of the organism profile and antibiotic susceptibility pattern of patients who were diagnosed with diabetic foot ulcers between January 2023 and December 2023 will be carried out at the Malabar Medical College Hospital and Research Centre (MMCH & RC). Data will be tracked from the medical records and data management system based on the exclusion and inclusion criteria and by using the self-designed Proforma. Specimens will be cultured using optimal standard microbiological techniques. The patient's pus, tissue, swab, and whole blood will be cultured. Identification of bacterial isolates will be performed using an automated culture identification and sensitivity testing system (VITEK). Microbiological methods and antibiotic susceptibility testing will be performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Anaerobic culture will not bedone due to the lack of resources for handling anaerobic samples. Hence, results will be analyzed for aerobic flora only.

Data analysis

Data was entered in Microsoft Excel, and data analysis was done using SPSS version 20. Numerical variables may be presented as mean +/- standard deviation. Categorical variables such as frequency and percentage. A chi-square test was used to test the association between multidrug-resistant organisms and variables. A P value of less than 0.05 will be considered statistically significant.

Results and Discussions

The present study included 106 patients with diabetes, and of these patients, 42 (39.6%) were female and 64 (60.4%) were male. The age ranged from 32 to 96 years, with the mean age being 61.40 years. Baseline characteristics of the 106 diabetic foot ulcer patients taken for the study showed that 60.4% (64/106) were males and 39.6% (42/106) were females. Increased male prevalence has been reported in other studies. This may be due to higher levels of outdoor activity among males than females. 22.2% (24/106) of patients had a previous history of amputation. Patients with a history of uncontrolled DM (82 [77%]) were more prone to diabetic foot infection. In our study, we found that most of the patients who were diagnosed with DFU had HbA1c values >6.5 ([85(80%]).

A total of 134 bacteria were isolated from these 106 patients. Of the 106 specimens from the diabetic foot lesions, culture showed polymicrobial growth in 34.9% (37/106), monomicrobial growth in 50.9% (54/106), and no growth in 14.2% (15/106). Microbiological evaluation of diabetic foot ulcer infections showed that the prevalence of Gram-negative organisms (79.10%, 106/134) was found to be more than Gram-positive organisms (20.89%, 28/134). Table 2 lists the organisms that were isolated from the DFIs. *Klebsiella species* was the most frequent pathogen (19.40% [26/134]), followed by *Proteus Group* [18% [24/134]) and *P. aeruginosa* (17.16% [23/134]). *Staph aureus* (13 [46.42%]) isolates were the bacterial species most commonly isolated among the Gram-positive bacteria, followed by *E. faecalis* [11 (35.71%) isolates] and *Staph haemolyticus (CoNS)* [4 (14.28%) isolates]. The antimicrobial susceptibility pattern of the Gram-positive and Gram-negative organisms is shown in Table 3.

The most prevalent gram-negative organism isolated was *Klebsiella species*. *Klebsiella species* showed susceptibility to cefuroxime, meropenem, amikacin, tigecycline, and cotrimoxazole. *Proteus Group* were susceptible to ceftriaxone, piperacillin tazobactam, meropenem, ciprofloxacin, gentamicin, and cotrimoxazole. *P. mirabilis* showed 100% susceptibility to cefoperazone sulbactam. Most of the *P. aeruginosa* were susceptible to piperacillin-tazobactam, meropenem, amikacin, and cefoperazone sulbactam. The majority of *Acinetobacter baumannii* were susceptible to piperacillin-tazobactam, meropenem, gentamicin, tigecycline, and cotrimoxazole while being resistant to amoxicillin and clavulanic acid, cefuroxime, and levofloxacin. Similarly, *Enterobacter Aerogenes* showed 100% resistance to amoxicillin clavulanic acid and levofloxacin. Gram-negative cocci, like *Enterobacter Aerogenes* isolate, were 100% (3/3) susceptible to piperacillin-tazobactam, ceftriaxone, meropenem, ciprofloxacin, gentamicin, tigecycline, and cotrimoxazole. However, they showed varying susceptibility to cefuroxime and cefixime. The majority of isolates of *E. coli* and *K.Pneumoniae* were susceptible to amikacin, piperacillin-tazobactam, and imipenem. *S. fonticola* was fully resistant to amoxicillin, clavulanic acid, cefuroxime, and ceftazidime while being sensitive to piperacillin-tazobactam, cefoperazone-sulbactam, meropenem, tigecycline, and cotrimoxazole.

Staph aureus was the most prevalent gram-positive organism isolated (17 [60.71%]). Of the *Staph* isolates, 13 isolates were *Staph aureus* and 4 were *Staph haemolyticus (CoNS)*. *Staph aureus (MSSA)* isolates were 100% sensitive to vancomycin, gentamicin, tigecycline, cotrimoxazole and daptomycin; and totally resistant to ceftriaxone, cefuroxime, imipenem, and meropenem. *Staph aureus MRSA* was intrinsically resistant to all the beta-lactam group of antibiotics and carbapenem antibiotics. *MRSA* was resistant to all the antibiotics except ciprofloxacin, tigecycline, cotrimoxazole, rifampicin, daptomycin and vancomycin. *Staph haemolyticus (CoNS)* showed 100% sensitivity to tigecycline, vancomycin, levofloxacin, daptomycin and linezolid. *Staph haemolyticus (CoNS)* was 100 % susceptible to ampicillin, tigecycline, vancomycin clindamycin, teicoplanin, ceftaroline, and erythromycin. *E. faecalis* isolates were sensitive to ampicillin, tigecycline, vancomycin, teicoplanin, daptomycin and linezolid. *E. faecalis* isolates were 100% susceptible to linezolid and daptomycin.

The purpose of this study was to analyze the organism profile and pattern of antibiotic susceptibility in individuals with diabetic foot. DFU was shown to be more prevalent in men (64/ 60.4%) than in women (42/ 39.6%), Males are more likely to have DFIs because they engage in outdoor activities more frequently and are more likely to develop injuries and foot ulcers. These findings align with earlier research.[6,7]

Globally, the isolates found in DFI differ significantly. Most diabetic foot infections are known to be polymicrobial.[8] The findings matched those of other studies conducted in Mumbai, India (55.7%)[9], South

India (60%)[10], Italy (40%) [11] and Portugal (83.7%).[12] But according to some reports, the prevalence of monomicrobial infections are higher.[13,14] In our study greater percentage of monomicrobial infections was found (50.9%; 54/106).

The current study shows that, in accordance with other research,[8] the prevalence of Gram-negative organisms (79. 10%, 106/134) was greater than that of Gram-positive organisms (20. 89%, 28/134).

Interestingly, in our current analysis we found a significant trend of *K.Pneumoniae*, *Proteus species* and *P.aeruginosa* are among the gram-negative bacteria frequently associated to these ulcers same as identified by Atlaw A *et al.,*[15] One more common bacterium is the gram-positive Staphylococcus aureus.[16] Most studies show that Staphylococcus aureus is the most common [17,18,] but some focus on *K.Pneumoniae*.[19] These results are in line with the patterns found in the current study.

Gram-negative rods, like *Klebsiella species*, *Proteus species* and *P.aeruginosa* are increasingly prevalent, according to studies by Baral *et al.,* and Mohanty *et al.,*[20,21]Accordingly, we found that the most commonly isolated Gram-negative bacteria were *Klebsiella species* (19.40%) followed by *Proteus species* (18%) and *P.aeruginosa* (17.16%). Most of the pathogens were Gram-positive, with *S.aureus* (46.42%) being the most common and *E. faecalis* (35.71%) being the most frequent agent.

While some research focus on *K.pneumoniae*, [20] and *P.aeruginosa* (22,23), the majority of studies show the highest frequency of *Staph aureus* (6,24,25) these results are consistent with the patterns noted in the current investigation. Although the exact proportions may differ from study to study, they all highlight how crucial both gram-positive and gram-negative bacteria are to DFU.

These findings about *K.pneumoniae* prevalence point to regional diversity that is most likely caused by microbiological habitats and medical procedures like sample collecting and processing. Other Gram-negative cocci, such as *Klebsiella species*, *E.coli*, and *P. mirabilis*, were found to be 100% susceptible to piperacillin-tazobactam, ceftriaxone, imipenem, meropenem, amikacin, ciprofloxacin, gentamicin, tigecycline, and cotrimoxazole in previous studies. (26,27)

The most effective antibiotic against *P.aeruginosa*, according to our research, was piperacillin-tazobactam, amikacin, cefoperazone sulbactam and meropenem. Amikacin and piperacillin/tazobactam were identified by Paterson *et al.,* (2005) as the active agents against Pseudomonas(28,29) while *Acinetobacter Baumannii* showed Susceptibility to ciprofloxacin, aminoglycosides, TMP SMX, and Cephalosporins were noticed by Kassam *et al.,*[30]

In our study piperacillin-tazobactam, meropenem, gentamicin, tigecycline, and cotrimoxazole were susceptible to *Acinetobacter Baumannii* and totaly resistant to amoxicillin and clavulanic acid, cefuroxime, and levofloxacin.

The results were similar to another study performed in the hospital in Kuwait. *P.mirabilis* and *K. pneumoniae* highly sensitive to amikacin, cefoperazone/sulbactam were the most effective antibiotics for Gram-negative bacteria. Similarly, most of our *P.mirabilis* showed100% susceptibility to cefoperazone sulbactam, piperacillin-tazobactam, cefuroxime, ceftriaxone, and meropenem.(27)As in our study, many research reports different resistance patterns of *P.aeruginosa*, *P. vulgaris*, and *M. morganii* exhibit varying resistance to commonly used antibiotics such as levofloxacin, cefuroxime, and amoxicillin-clavulanic acid.(31)

Numerous studies have voiced concerns regarding the emergence of novel *S.Aureus* strains and the growing global health problem of antibiotic resistance. These findings demonstrated that while gentamicin, vancomycin, tigecycline, cefuroxime, and daptomycin showed potential in treating MSSA infections.While, ciprofloxacin, tigecycline, cotrimoxazole, rifampicin, daptomycin and vancomycin were effective against *S. aureus* infections (MRSA). These findings are consistent with a prior report from a retrospective study published in the Saudi Journal of Biological Sciences, which found that MRSA and *S. aureus* were 100% sensitive to gentamicin and tigecycline. (32)

As a potentially cost-effective antimicrobial medication for treating MRSA infections, Co-trimoxazole may be preferred in light of our current study as well as other research by Bishara J *et al.,* and Elwell LP *et al.,* (33,34) From our finding, *S. haemolyticus* shows 100% sensitivity to tigecycline, linezolid, levofloxacin and vancomycin, as the same as the previous study done from South China.(35)

Table.1 Characteristics of the DFU patients

PARAMETERS	**VALUES**
Mean (SD) Age	61.40
SEX	
Male	64(60.4%)
Female	42(39.6%)
Duration of diabetes (years)	14.65
Duration of hospital stay (days)	8.20
LESIONS INVOLVED	
Left foot	46(43.4%)
Right foot	55(51.9%)
Both feet	5(4.7%)
PREVIOUS HISTORY OF AMPUTATION	
Yes	49(46.2%)
No	57(53.8%)
NATURE OF MICROBIAL GOWTH	
No growth	15(14.2%)
Monomicrobial	54(50.9%)
Polymicrobial	37(34.9%)
DIABETES MELLITUS TYPE	
Controlled	24(23%)
Uncontrolled	82(77%)
Hba1c VALUE	
≤6.5	21 (20%)
>6.5	85(80%)
STAGE OF DIABETIC FOOT	
GRADE 1	4(3.8%)
GRADE 2	36(34%)
GRADE 3	40(37%)
GRADE 4	20(18.8%)
GRADE 5	6(5%)

Table.2 Bacteria isolated from diabetic foot ulcers

Sl No.	**Bacteria**	**No of isolates (%)**
1.	*Klebsiella species*	26
2.	*Proteus Group*	24
3.	*P. aeruginosa*	23
4.	*E. coli*	16
5.	*Acinetobacter baumannii*	13
6.	*E. faecalis*	11
7.	*Staph aureus(MRSA)*	10
8.	*S. fonticola*	4
9.	*Staph haemolyticus (CoNS)*	4
10.	*Staph aureus (MSSA)*	3

Table.2 Bacteria isolated from diabetic foot ulcers

Antibiotic susceptibility pattern obtained from Gram-positive bacteria		**Total no: of Gram-Positive bacteria obtained from Diabetic foot isolates**		
		E. faecalis (n=11)	***Staph aureus (n=13)***	***Staph haemolyticus (CoNS) (n=4)***
Antibiotic sensitivity	**Ampicillin**	9 (81.8%)	3 (100%)	1 (25%)
	Tigecyline	10 (90.9%)	13 (92.3%)	4 (100%)
	Co-trimoxazole		13 (92.3%)	3 (75%)
	Clindamycin		9 (69.2%)	3 (75%)
	Vancomycin	8 (72.7%)	13(100%)	4 (100%)
	Pencillin	8 (72.7%)	1 (33.3%)	4 (100%)
	Levofloxacin	1 (9%)	8 (61.5%)	4 (100%)
	Linezolid	11 (100%)	9 (69.2%)	4 (100%)
	Teicoplanin	10 (90.9%%)	10(76.9%)	3 (75%)
	Rifampicin		9 (69.2%)	1 (25%)
	Oxacillin		11(84.6%)	1 (25%)
	Daptomycin	11 (100%)	13(100%)	4 (100%)
	Tetracycline	1 (9%)	7(53.8%)	3 (75%)
	Minocycline		1 (33.3%)	
	Ciprofloxacin	1 (9%)	7(53.8%)	1 (25%)
	Gentamicin	3 (27.2%)	9 (69.2%)	3 (75%)
	Erythromycin		5 (38.4%)	3 (75%)

Table.3 Bacteria isolated from diabetic foot ulcers

Antibiotic susceptibility pattern obtained from Gram-negative bacteria		**Total no: of Gram-Negative bacteria obtained from Diabetic foot isolates**							
		Acinetobacter Baumannii (n=13)	*Enterobacter Aerogenes* (n=3)	*E.coli* (n=16)	*K.Pneumoniae* (n=23)	*M. morganii* (n=12)	*P. mirabilis*(n=12)	*P. aeruginosa* (n=23)	*Serratia Fonticola* (n=4)
Antibiotic sensitivity	Amoxicillin/ clavulanic acid.			10 (62.5%)	9 (39.1%)		5 (41.6%)		
	Piperacillin/ tazobactam	2 (15%)	3 (100%)	11 (68.7%)	9 (39.1%)	10 (43.4%)	11 (91.6%)	13 (56.52%)	3 (75%)
	cefuroxime		1 (33.3%)	3 (18.7%)	9 (39.1%)	1 (8.3%)	3 (25%)		
	Ceftriaxone/ cefotaxime	1 (7.70%)	3 (100%)	3 (18.7%)	8 (34.7%)	5 (41.6%)	7 (58.3%)		2 (50%)
	Cefoperazone/ sulbactam	3 (23%)	2 (66.6%)	10 (62.5%)	10 (43.4%)	10 (43.4%)	12 (100%)	14 (60.8%)	3 (75%)
	Cefepime	3 (23%)	2 (66.6%)	6 (37.5%)	9 (39.1%)	2 (8.6%)	7 (58.3%)	13 (56.5%)	2 (50%)
	Imipenem	3 (23%)	3 (100%)	11 (68.7%)	9 (39.1%)		4 (33.3%)	14 (60.8%)	2 (50%)
	Meropenem	3 (23%)	3 (100%)	10 (62.5%)	11 (47.8%)	8 (66.6%)	11 (91.6%)	14 (60.8%)	3 (75%)
	Amikacin	2 (15%)	3 (100%)	13 (81.2%)	14 (60.8%)	10 (43.4%)	9 (75%)	16 (69.5%)	3 (75%)
	Ciprofloxacin	1 (7.70%)	3 (100%)	2 (12.5%)	8 (34.7%)	5 (41.6%)	7 (58.3%)	12 (52.1%)	2 (50%)
	Gentamicin	3 (23%)	3 (100%)	10 (62.5%)	14 (60.8%)	8 (66.6%)	9 (75%)	8 (34.7%)	3 (75%)
	Tigecyline	10 (76.9%)	3 (100%)	16 (100%)	10 (100%)			1 (4.34%)	2 (50%)
	Co-trimoxazole	4 (30.7%)	3 (100%)	9 (56.2%)	14 (60.8%)	8 (66.6%)	7 (58.3%)	2 (8.6%)	1 (25%)
	Ceftazidime	1 (7.70%)						14 (60.8%)	
	Levofloxacin							10 (43.4%)	1 (25%)
	Minocycline	5 (38.4%)							

Table.4 Significance between variables

Variables		Type of Aerobe				Chi-Square Fisher's Exact Test	P Value	Significance
		Gram +Ve	Gram _Ve	Gram +Ve & Gram _Ve	Blank/No Growth			
Previous History of Amputation	Yes	3(6.1%)	38(77.6%)	2(4.1%)	6(12.2%)	8.133	.041	>0.05 significance
	No	11(19.3%)	30(52.6%)	7(12.3%)	9(15.8%)			
Gender Group	Female	2 4.8%	33 78.6%	4 9.5%	3 7.1%	8.513a	.037	>0.05 significance
	Male	12 18.8%	35 54.7%	5 7.8%	12 18.8%			

All the organisms stated above shown resistance to different kinds of antibiotics. However, the pattern of resistance varied considerably according to antibiotic use, microorganism prevalence, and geography. It is recommended that we ensure the proper usage of antibiotics in order to combat antibiotic resistance.

In conclusion, the current study shows that the prevalence of Gram-negative organisms was greater than that of Gram-positive organisms. DFU was shown to be more prevalent in men than in women. In our study, a greater percentage of monomicrobial infections was found. The DFU was significantly higher in patients with uncontrolled diabetes. Klebsiella species were the most common microorganisms found in the diabetic foot ulcer patients. Amikacin, meropenem, and gentamicin were found to be beneficial for Gram-negative pathogens, whereas daptomycin, tigecycline, vancomycin, and linezolid were found to be helpful against Gram-positive agents.

According to the antimicrobial susceptibility data from our investigation, daptomycin seems to be the best choice for empirical coverage of gram-positive pathogens, while amikacin would be the greatest choice for gram-negative coverage. Diabetic foot infections had a strikingly high frequency of MDR pathogens. Clinical recommendations must be adhered to to enhance patient outcomes and lower the prevalence of MDR bacteria in this population.

The study emphasizes the need for preventing the DFI by stricter diabetic control measures and rational use of antibiotics with correlation to organisms and their susceptibility pattern.

Author Contributions

V. V. Megha: Investigation, formal analysis, writing—original draft. Aksa Merin Jose: Validation, methodology, writing—reviewing. M. K. Dinesh Kumar:—Formal analysis, writing—review and editing. T. S. Shailaja: Investigation, writing—reviewing. M. P. Sasi: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Megha V. V., Aksa Merin Jose, Dinesh Kumar M. K., Shailaja T. S. and Sasi M. P. 2025. Organism Profile and Antibiotic Susceptibility Pattern Analysis of Diabetic Foot Patients of a Rural Tertiary Care Hospital in South India. *Int.J.Curr.Microbiol.App.Sci.* 14(11): 337-345. **doi:** https://doi.org/10.20546/ijcmas.2025.1411.035