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An Analysis of Bacterial Isolates from Pus Culture Versus Tissue Culture in Diabetic Foot Infection Through Amit Jain's Classification for Diabetic Foot Complications

Amit Kumar C. Jain[1,2*], H. C. Apoorva[1] and Kishore Kumar[1]

[1]Amit Jain's Diabetic Foot and Wound Research Unit, Amit Jain's Institute of Diabetic Foot and Wound Care, Brindhavvan Areion Hospital, Bengaluru, India.
[2]Department of Surgery, Raja Rajeswari Medical College, Bengaluru, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AKCJ designed the study and wrote the manuscript and was in involved in statistical analysis. Author HCA collected the data and wrote the first draft of manuscript. Author KK did the critical analysis and revision. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To compare pus culture versus tissue culture for bacterial isolates of diabetic foot infections through Amit Jain's classification for diabetic foot.
Materials and methods: A retrospective comparative analysis was done at Amit Jain's Institute of Diabetic foot and Wound Care, BrindhavvanAreion hospital, Bengaluru. The study period was from Jan 2019 to June 2020. Statistical Analysis was done using SPSS version 25.0 and p value of < 0.05 was considered significant.
Results: 42 patients were included in this study. Tissue culture yielded bacteria in 90.5% of cases. Pus culture had multiple growths in 9.5% whereas tissue culture had multiple growths in 16.7%. In both pus and tissue cultures, Gram negative organisms were common compared to Gram positive

**Corresponding author: E-mail: dramitkumarcj@yahoo.in;*

and *E* coli was commonest isolate in both. No difference was seen in type 1 and type 3 diabetic foot complications in regards to number and type of bacterial isolates in pus culture. Whereas in tissue culture, significant difference was noted wherein type 1 diabetic foot complication had more Gram positive organisms and type 3 diabetic foot complications had Gram negative organisms. In type 1 diabetic foot complications, pus culture had 77% sensitivity and 100% specificity and was comparable to tissue culture.

Conclusion: Tissue culture was found to have higher yield and higher percentage of multiple organisms compared to pus culture. However, in type 1 diabetic foot complications like abscess, wet gangrene, necrotizing fasciitis, etc,one can rely on swab culture as its yields were comparable with tissue culture. We believe that pus culture is a reasonable choice for bacterial isolate in diabetic foot complications.

Keywords: Diabetic foot; pus; tissue; culture; classification; diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus is a major global health problem of 21st century [1,2,3]. It had 285 million people affected in 2009 and increased to 463 million, today [3]. Developing country like India, with large population, has around 33 million diabetics [1].

One feared complication of diabetes is diabetic foot [1,3]. It is estimated that 15-25% of diabetic patients will develop ulcer in foot during their lifetime [4,5]. More than half of these foot ulcers will become infected [1]. Infections in the foot leads to increased morbidity and may lead to amputation [5]. There are multiple reasons to it like walking barefoot, illiteracy and poor foot care [5].

Diabetic foot ulcer often precedes in 85% cases of lower extremity amputation [6]. Most of the infections are of mixed microbiology [4]. Approximately 20% of infected ulcers may end up in some amputation [7]. Hence, once infections set in, need for surgical intervention and use of antibiotics become essential. Culture plays essential role in identifying the causative organism.

Swab culture and tissue culture are 2 ways of obtaining infectious etiology information and often tissue culture is considered to be better than swab culture as it avoids contaminants [8]. However, there is disparity among experts with few finding swab culture to be an acceptable and valuable option for assessing microbiology of diabetic foot [9,10].

For years, the swabs versus tissue culture studies were done predominantly for ulcers of foot [9,10]. They were often studied through Wagner's classification and also PEDIS classification [10,11]. These are ulcer classifications which is a focal entity. Recently, a new universal classification for diabetic foot was proposed from India for first time for diabetic foot [12,13]. This classification includes all lesions seen in diabetic foot worldwide [12] and it basically divided diabetic foot into 3 types (Table 1). We aimed to conduct a comparative study between pus culture and tissue culture through this new classification from India [12].

Table 1. Showing the Amit Jain's universal classification for diabetic foot complications

Type of diabetic foot complications	Pathological Lesions
Type 1 diabetic foot complications (Infective)	Abscess, wet gangrene, cellulitis, necrotizing fasciitis, etc
Type 2 diabetic foot complications (non-Infective)	Tropic ulcer, dry gangrene, Charcot foot, hammer toe, etc
Type 3 diabetic foot complications (when type 2 complications get infected)	Example- Trophic ulcer with osteomyelitis, dry gangrene with secondary infection, infected Charcot foot, etc

2. MATERIALS AND METHODS

A descriptive comparative analysis was done at Amit Jain's Institute of Diabetic Foot and Wound Care, Brindhavvan Areion Hospital, Bengaluru, India. The study period was from Jan 2019 to June 2020. We studied the pus and tissue cultures in patients with diabetic foot infections. The pus is normally collected in a sterile swab and sent to microbiology laboratory. The deep infected tissue is normally obtained by us intra-operatively with sterile instrument (Fig. 1) and is sent to microbiology laboratory in a sterile container containing normal saline. The pus and the tissue specimen undergo gram staining and are inoculated onto the MacConkey agar, blood agar and chocolate agar to look for any aerobic growth at 37^0C.

2.1 Statistical Analysis

Descriptive statistics were reported as mean and SD, number and percentages. Independent t test was used to compare the age by type of foot complications and surgery done. Chi-square test was used to test the association between study variables. Analysis was carried out separately for pus and tissue culture. Association between type of bacterial isolates and number of isolates with type of foot complications and surgery done was carried out using chi-square test or Fisher's exact test as appropriate. P value <0.05 was considered statistically significant. All the analysis was done using SPSS version 25.0.

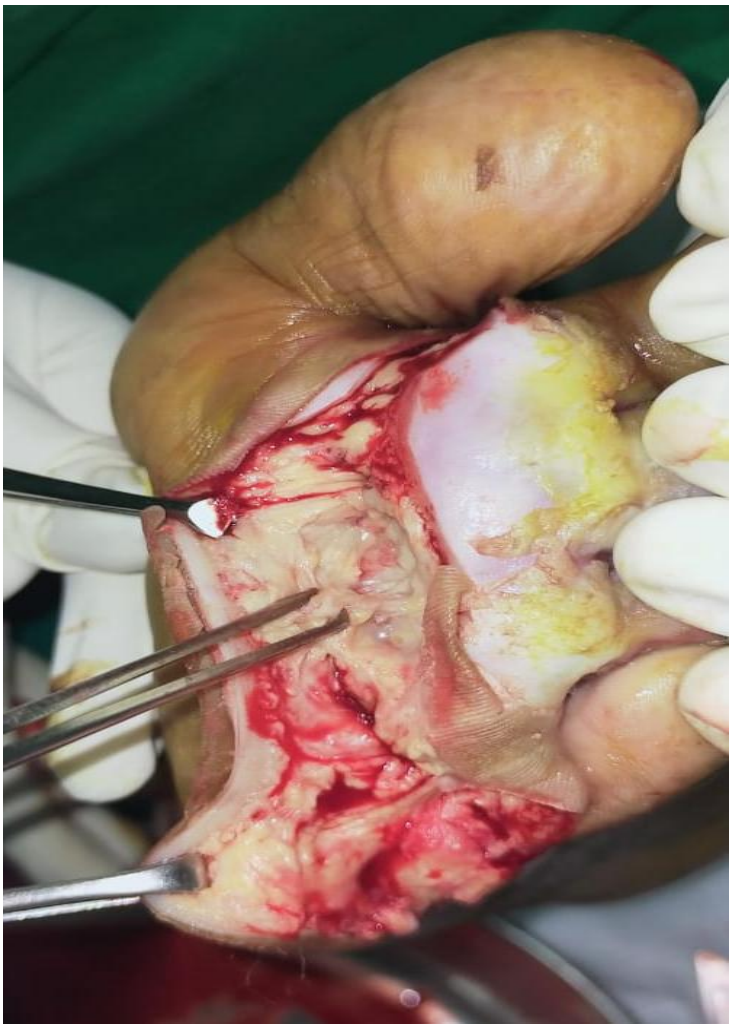

Fig. 1. Showing deep infected tissue obtained intra-operatively in a diabetic foot infection that is sent for culture

3. RESULTS

A total of 42 cases were included in the study. The mean age of the patients studied was 57.6 ± 9.13 years having 76% of males. Type of foot complications and surgery done were comparable between male and female. Mean age of the patients were comparable by type of foot complications and surgery done. In pus culture (group 1), 66.7% had some growth whereas in tissue culture (group 2), 90.5% of the cases had some growth. In pus culture, 38.1% had gram negative bacteria whereas in tissue culture, 61.9% had gram negative organism. Pus culture had 57.1% single isolate and tissue culture had 73.8% as single isolate (Table 2).

Commonest bacteria isolated in pus culture(group 1) was *E*.coli and MRSA accounting for 14.3% each (Table 3), whereas the commonest bacteria isolated in tissue culture was *E*.coli (23.8%) followed by Klebsiella sp (14.3%).

Gram positive and gram negative bacterial isolates were equal in type 1 diabetic foot complications (Table 4). Whereas gram negative bacteria (39.1%) was more frequently isolated than gram positive in type 3 complications in pus culture group and there was no statistical difference (P- 0.507). In tissue culture group, gram positive bacteria(52.6%) was more commonly isolated in type 1 diabetic foot complications and gram negative (78.3%) bacteria was more commonly isolated in type 3 diabetic foot complications and it was significant (*P- 0.007*). There was no significant difference in bacterial isolates in both groups.

Table 2. Showing presence of growth of bacteria in two groups

Clinical variables	Groups studied	
Growth of Bacteria	Group 1(Pus culture)	Group 2(Tissue culture)
Growth present	28 (66.7%)	38 (90.5%)
Growth Absent	14 (33.3%)	4 (9.5%)
Type of Bacterial Growth		
Gram Positive	12 (28.6%)	12 (28.6%)
Gram Negative	16 (38.1%)	26 (61.9%)
No Growth	14 (33.3%)	4 (9.5%)
Number of Bacterial Isolates		
Single	24 (57.1%)	31 (73.8%)
Multiple	4 (9.5%)	7 (16.7%)
No Growth	14 (33.3%)	4 (9.5%)

Table 3. Showing the organisms that were isolated in the two groups

Type of organisms	Group 1	Group 2
Acinetobacter baumannii	1 (2.4)	3 (7.2)
Enterococci	2 (4.8%)	3 (7.2%)
Escherichia coli	6 (14.3%)	10 (23.8%)
Klebsiella pneumonia	4 (9.5%)	6 (14.3%)
MRSA	6 (14.3%)	5 (11.9%)
Morganella morganii	1 (2.4%)	2 (4.8)
Proteus mirablilis	1 (2.4%)	0(0%)
Proteus vulgaris	2 (4.8%)	1 (2.4%)
Pseudomonas aeruginosa	1 (2.4%)	1 (2.4%)
Vancomycin resistant enterococci	1 (2.4%)	1 (2.4%)
Citrobacter freundii	0 (0%)	1 (2.4%)
Enterobacter	0(0%)	1 (2.4%)
MSSA	3 (7.2%)	2 (4.8%)
No growth	14 (33.3%)	4 (9.5%)
Total	42 (100%)	42 (100%)

Table 4. Showing comparison of clinical variables between the two groups and type of diabetic foot complications

Groups	Variables	Type of diabetic foot complications		Total	P value
		Type 1 Diabetic foot complications	Type 3 diabetic foot Complications		
Group 1(Pus culture)	Type of bacterial isolates				
	Gram positive	7 (36.8%)	5 (21.7%)	12 (28.6%)	
	Gram negative	7 (36.8%)	9 (39.1%)	16 (38.1%)	0.507
	No growth	5 (26.3%)	9 (39.1%)	14 (33.3%)	
	Number of isolates				
	Single	13 (68.4%)	11 (47.8%)	24 (57.1%)	
	Multiple	1 (5.3%)	3 (13)	4 (9.5%)	0.378
	No growth	5 (26.3%)	9 (39.1%)	14 (33.3%)	
Group 2(Tissue culture)	Type of bacterial isolates				
	Gram positive	10 (52.6%)	2 (8.7%)	12 (28.6%)	
	Gram negative	8 (42.1%)	18 (78.3%)	26 (61.9%)	0.007
	No growth	1 (5.3%)	3 (13%)	4 (9.5%)	
	Number of isolates				
	Single	16 (84.2%)	15 (65.2%)	31 (73.8%)	
	Multiple	2 (10.5%)	5 (21.7%)	7 (16.7%)	0.376
	No growth	1 (5.3%)	3 (13%)	4 (9.5%)	

Table 5. Showing comparison of types of bacterial growth with number of isolates between the two groups

Groups	Types of bacterial growth	Number of isolates			Total	P value
		No growth	Single	Multiple		
Group 1	No growth	14 (100%)	0 (0%)	0 (0%)	14 (33.3%)	<0.001
	Gram positive	0 (0%)	9 (37.5%)	3 (75%)	12 (28.6%)	
	Gram Negative	0 (0%)	15 (62.5%)	1 (25%)	16 (38.1%)	
Group 2	No growth	4 (100%)	0 (0%)	0 (0%)	4 (9.5%)	<0.001
	Gram positive	0 (0%)	10 (32.3%)	2 (28.6%)	12 (28.6%)	
	Gram negative	0 (0%)	21 (67.7%)	5 (71.4%)	26 (61.9%)	

It was however seen that in group 1, 75% of multiple growth had gram positive bacteria, whereas in group 2, 71.4% of multiple isolates had gram negative (Table 5) and it was statistically significant (*P-<0.001*).

There was no association of number of isolates or type of bacterial isolate with type of surgery done in group 1. Whereas a significant association of type of bacterial isolate was seen with type of surgery done in group 2 (Table 6). Single growth was more common in debridement and minor amputation (*P-0.027*). Gram negative bacteria (69.6%) were commonly isolated in patient who underwent debridement (*P-0.03*).

Overall, the pus culture in comparison to tissue culture, had a sensitivity of 68%, specificity of 50% with PPV of 93% and it was statistically significant (*P-0.013*).

However, it was noted that in type 1 diabetic foot complications the pus culture had 77% sensitivity, 100% specificity and 100% positive predictive value and was comparable to tissue culture group. In type 3 diabetic foot complication, the pus culture had 60% sensitivity and 33% specificity compared to tissue culture.

4. DISCUSSION

Diabetic foot infections are limb threatening condition and they are often polymicrobial in

Table 6. Showing comparison of bacterial isolates with type of surgery done between the two groups

Groups	Variables	Type of surgery done			Total	P value
	Number of isolates	**Debridement**	**Minor amputation**	**Major amputation**		
	No growth	7 (30.4%)	7 (38.9%)	0 (0%)	14 (33.3%)	
	Single	14 (60.9%)	9 (50%)	1 (100%)	24 (57.1%)	0.869
	Multiple	2 (8.7%)	2 (11.1%)	0 (0%)	4 (9.5%)	
Group 1 (Pus Culture)	Type of bacterial isolates					
	No growth	7 (30.4%)	7 (38.9%)	0 (0%)	14 (33.3%)	
	Gram positive	8 (34.8%)	4 (22.2%)	0 (0%)	12 (28.6%)	0.648
	Gram negative	8 (34.8%)	7 (38.9%)	1 (100%)	16 (38.1%)	
	Number of isolates					
	No growth	1 (4.3%)	2 (11.1%)	1 (100%)	4 (9.5%)	
	Single	17 (73.91%)	14 (77.8%)	0 (0%)	31 (73.8%)	0.027
	Multiple	5 (21.7%)	2 (11.1%)	0 (0%)	7 (16.7%)	
Group 2 (Tissue culture)	Type of bacterial isolates					
	No growth	1 (4.3%)	2 (11.1%)	1 (100%)	4 (9.5%)	
	Gram positive	6 (26.1%)	6 (33.3%)	0 (0%)	12 (28.6%)	0.03
	Gram negative	16 (69.6%)	10 (55.6%)	0 (0%)	26 (61.9%)	

nature consisting of mixed aerobic and anaerobic organisms often leads to increased hospital stay and treatment cost apart from morbidity and risk of morbidity from sepsis [14].

Recurrent ulcers, large ulcers and long duration ulcers often harbor multidrug resistance isolates [4,6].

Open wounds are often prone to contamination and thus when obtaining culture, often it is advised to clean and irrigate it with saline [15].

There is a geographic variation in organism isolated from diabetic foot. Some reports from western literature found gram positive organism to be predominant whereas some studies in India found gram negative organism to be predominant [6].

Studies have also been done between swab culture and tissue culture especially on ulcers and tissue culture has been often advised over swab culture [10,11]. Though tissue culture may be considered to be best method to identify organism it is invasive in nature [11]. However, in most part of the world, swab cultures are commonly employed in view of its non invasive nature and ease to do [10]. It can also be done by any healthcare worker especially in primary care setups [10]. Huang et al found no difference in the mean number of isolates between swab culture and tissue culture in diabetic foot ulcers [11]. Another study noted that 62% of cases swab and tissue culture yielded some organism and 18% of tissue culture identified more organisms [8]. These were diabetic foot ulcers.

In our study, through Amit Jain's universal classification for Diabetic foot which has variety of lesions like abscess, gangrene, necrotizing fasciitis, ulcers, etc, the type 1 Diabetic foot complications (acute) had gram positive organism to be common in tissue cultures, where as type 3 diabetic foot complications (acute on chronic) had grown negative organism to be predominant. There was as such no difference in pus culture and tissue culture in regards to bacterial growth. In Sankar et al series, swab culture yielded 86.07% of bacteria whereas tissue culture yielded 97.47%. in our study, pus culture yielded bacterial growth in 66.7% and in tissue culture, bacterial growth was seen in 90.5% [9]. Macis et al suggests swab culture to

be a sensible method [9,16]. Another study suggested swab cultures to be valuable in identifying organisms in diabetic foot when bone is uninvolved [10].

The debate continues whether swab culture is better or tissue culture is better in diabetic foot ulcers with each having their own merits and demerits. Though tissue culture is preferred over swab culture in identifying more organisms, the swab culture is more commonly done due to ease and non invasive nature. In this study, a comparison was done between pus culture and tissue culture through a new universal classification that has numerous lesion and not just ulcers. This study shows that in type 1 diabetic foot complication, which is acute in nature, pus culture had 77% sensitivity and 100% specificity and was comparable to tissue culture. Whereas in type 3 diabetic foot complications (acute and chronic), the pus culture had low sensitivity and specificity compared to tissue culture and in such cases through initially swab culture cab be done it should always have tissue culture also, especially obtained during surgery for better yield of organism and antibiotic therapy course post operatively. Further studies are needed in this field

5. CONCLUSION

Tissue culture was found to have higher yield and higher percentage of multiple organisms compared to pus culture overall. However, in type 1 diabetic foot complications like abscess, wet gangrene, necrotizing fasciitis, etc, one can rely on swab culture as its yields were comparable with tissue culture. We believe that pus culture is a reasonable choice for bacterial isolate in Type 1 diabetic foot complications. Further studies are needed in this regard.

CONSENT

Not applicable, chart based study.

ETHICAL APPROVAL

An institutional ethics committee approval was obtained for this study (RRMCH-IEC/12/2020-21)

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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