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Original Research Article

Comparative Study of Standard Loop Technique and Pour Plate Technique in Identifying Significant Bacteriuria

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Abstract

Objectives: The aim of this study is to compare two different methods in identifying significant bacteriuria. ***Materials and Methods:*** A cross-sectional study was conducted for a period of three months from January to March 2019. Urine samples received to Clinical Microbiology Laboratory were subjected to Standard loop technique and Pour plate technique.10^4 CFU/ml was considered as significant bacteriuria. Statistical analysis was made by chi-square test. ***Results:*** Out of 300 urine samples, 87 samples were shown to have Significant Bacteriuriaby Standard loop technique, 91 samples were shown to have Significant Bacteriuria by Pour plate technique. The Escherichia coli accounts for (30%) of isolates causing Significant Bacteriuria followed by Proteus species (17%), Enterococcus species (16%), and Pseudomonas species (14%). Male preponderance was observed over females. The Pour plate technique was found to be most sensitive method in identifying significant Bacteriuria. ***Conclusion:*** The Pour plate technique was observed to be the sensitive method in identifying significant bacteriuria compared to standard loop technique. *Escherichia coli* showed the highest rate of isolation [1]. Multi-drug resistance is seen in Klebsiella species.

Keywords: Standard loop technique, pour plate technique, significant bacteriuria.

INTRODUCTION

Bacteriuria is the presence of bacteria in urine. Bacteriuria accompanied by symptoms is called as significant bacteriuria while without is known as insignificant bacteriuria [2]. Insignificant bacteriuria generally does not require any treatment. Exceptions include undergoing surgery of urinary tract. Significant bacteriuria is synonymous and treated with antibiotics. The methods used in this study are standard loop and pour plate technique. The standard loop technique is a simplified method in estimating the total viable counts present in a sample [3]. The pour plate technique is usually the method of choice for counting the number of colony forming units in a liquid specimen.

MATERIALS AND METHODS

A cross sectional study conducted in the department of microbiology at saveetha medical college and hospital during the period of January to march 2019. Urine samples received to Clinical Microbiology Laboratory were included in this study after getting approval from Institutional review board. Samples were subjected to two different methods for analysing significant bacteriuria. Bacterial pathogens were isolated and identified by conventional methods. Antibiotic susceptibility testing was done for checking effective antibiotic to be prescribed [4].

Microscopic Examination

Gram staining is a method of staining used to distinguish and classify bacterial species into two large groups (gram positive and gram negative).

Culture Methods

Media used was Blood agar and Mac Conkey agar to isolate causative organisms from urine specimens [5]. They were incubated aerobically at 37°C for 24 to 48 hours.

Standard Loop Technique

In standard loop technique an caliberated inoculation loop with was used to take fixed and known volume of uncentrifuged urine and it was spread over a plate on agar culture medium. It can hold 0.002 ml urine. The number of colonies were counted and this number was used to calculate the number of viable

bacteria per ml of urine by following significant bacteriuria (kass concept).

(CFU) per ml of urine	Report the bacterial count
< 10 000 organisms/ml (10^4ml/)	Not significant
10 000 – 100 000/ml ($10^4 - 10^5$/ml)	Doubtful (repeat specimen)
>100 000/ml (10^5/ml)	Significant bacteriuria

Total viable bacterial count per ml sample = no. of colonies x 500

Pour Plate Technique

This method is used to count the number of microorganisms in a mixed sample, which is added to a molten agar medium prior to its solidification. The process results in colonies uniformly distributed throughout the solid medium when the appropriate sample dilution is plated. This technique is used to perform viable plate counts, in which the total number of colony forming units within the agar and on surface of the agar on a single plate is enumerated. Viable plate counts provide a standardised means to generate growth curves, to calculate the concentration of cells in the tube from which the sample was plated, and to investigate the effect of various environments on bacterial cell survival or growth rate [6]. The significant bacteriuria was calculated based on the above table.

Antibiotic Susceptibility Testing

Susceptibility pattern was done by disk diffusion method. All the isolated organisms subjected for antibiotic susceptibility test by kirby - bauer disc diffusion technique [7]. The tests were performed on muller - hinton agar plates. Sterile cotton swab stick was used to make a lawn culture. Inoculated plates were incubated at 37ºC for 24 hours.

RESULT

Urinary Tract Infection is the most common infection in developing countries. In this study, out of 300 urine suspected cases of urinary tract infection, 102 (34%) cases showed significant bacterial growth and 198 cases showed no growth. Male predominance is observed in this study [8].This is explained in Table-1. The organisms isolated from patients are *Escherichia coli* (30%), Proteus (17%), Pseudomonas (14%), Klebsiella (10%), Enterococcus (16%), coagulase negative staphylococcus species (cons) (9%), Burkholderia (1%). The reason of highest rate isolation of *Escherichia coli* causing urinary tract infection is due to the fact that most of bacterial organisms causing urinary tract infection originate from the fecal flora. In this study klebsiella showed more resistant to Gentamicin,cefuroxime,cefotaxime,cefepime,cefeperazone sulbactam. *Escherichia coli* was found to be most sensitive. Burkholderia showed least resistance to Amikacin (0%), Gentamicin (0%), Piperacillin tazobactum (0%), Cefeperazone sulbactam (0%). This is explained in Table-2.

Table-1: Age wise sex wise and Growth positivity of Urinary isolates

	Male		Female		Total
	IP	OP	IP	OP	
Growth	27	11	56	8	102
No growth	39	19	130	10	198
	66	30	186	18	300
Total	96		204		
Chi square for Male IP and OP for growth positive is 0.1552. P=0.693594. Not significant Chi square for Female IP and OP for growth positive is 1.5668. P=0.210669. Not significant Chisquare for IP and OP for growth positive is 0.7938. P=0.372945. Not Significant Chi square for Male and Female for growth positive is 1.9612. P=0.16138. Not significant					

Table-2: Percentage of Isolates resistant to antibiotics (Kirby bauer disc diffusion method)

Organism	No. of isolates	Ak	G	CFX	CTX	CPM	PIT	CFS	NIT
Klebsiella species	11	50	100	100	100	100	100	100	100
Escherichia coli	26	0	40	100	100	40	40	40	0
Burkholderia cepacia	6	0	0	100	100	0	0	0	100
Pseudomonas aeruginosa	15	33	33	100	100	33	66	100	100
Proteus species	18	25	25	75	100	100	50	100	100
Total isolate	76								
Organism	**No. of isolates**	**NIT**	**P**	**A**	**HLG**	**VA**	**LZ**		
Enterococcus species	17	66	66	0	66	0	0		
Coagulase negative Staphylococcus species	9	0	100	100	0	0	0		
Total isolate	26								
AK - Amikacin, G - Gentamicin, CFX - Cefuroxime, CTX - Cefotaxime, CPM - Cefepime, Pit - Piperacillin tazobactam, CFS - Cefeperazone sulbactam, NIT - Nitrofurantoin, P - Penicillin, A - Ampicillin, HLG - High level Gentamicin, VA - Vancomycin, LZ - Linezolid									

The Pour plate technique is found to be the effective method in identifying significant bacteriuria since it is found to be most sensitive. Explained in Table-3.

Table-3: Comparison between Standard loop technique and Spread plate technique

Method		**Spread plate technique**		**Total**
		Significant bacteriuria	Insignificant bacteriuria	
Standard loop technique	Significant bacteriuria	76	11	87
	Insignificant bacteriuria	15	198	213
	Total	91	209	300

The chi square statistic is 188.5453. The P value is <0.00001. The result is significant at P<0.05.

DISCUSSION

An ideal method for detecting significant bacteriuria is one that is simple, inexpensive, accurate, and convenient to use under the conditions requiring its use. It should also not require too much skill.

The Pour plate technique and standard loop technique are performed to identify the bacteriuria present in urine samples [9]. Out of 300 samples, 198 samples showed no growth where as 102 were growth positive. Male ip(in patient) and op (out patient) for growth positive is 0.1552, whereas for female patients is 1.5668.

The Pour plate technique identifies the true positive organisms which makes it more sensitive. Sensitivity is a measure of true positive rate [10]. It quantifies the avoiding of false negatives. It refers to the test's ability to correctly detect ill patients. It will give a lower count as heat sensitive microorganisms may die when they come contact with hot, molten agar medium. The true positive organisms identified in this study are Staphylococcus, Pseudomonas aeroginosa, Klebsiella, enterococcus. The Standard loop technique is less sensitive (specificity) because it may show the presence of false positive organisms.

Specificity refers to true negative rate [11]. It relates to the test's ability to correctly reject healthy patients without a condition. In loop technique, the risk of contamination might be present. The loop dipped into the agar medium may not be capable to hold the adequate volume of the sample and leads to increased negative results [12].

CONCLUSION

According to findings, pour plate technique was observed to be the most effective method in identifying significant bacteriuria. The most common isolated bacteria from urinary tract infections is Escherichia coli [13]. The effective antibiotics against gram negative bacteria are Cefuroxime, Cefotaxime. Whereas for gram positive bacteria the effective antibiotics are Gentamicin. Male predominance was observed. This study will guide the physicians to choose the pour plate technique over loop technique since it is more precise and not requiring previously prepared plates [14]. This ensures effective and quick treatment of the infection and preventing antibiotic resistance and better results in a tertiary care center.

Conflict of Interest: NIL

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