UNIVERSITI PUTRA MALAYSIA

EVALUATION OF TWEEN 80 INCORPORATED MEDIA TO INCREASE PATHOGEN ISOLATION FROM PERITONEAL FLUID OF CAPD PATIENTS AT A PUBLIC HOSPITAL IN KUALA LUMPUR, MALAYSIA

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By

STELLA A/P GANAPATHY PILLAY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2020

DEDICATION

In the Name of The Lord Almighty God

I dedicate this Thesis to my beloved husband Philip,

my adorable kids Prince Zechariah and Evelyn Zaharina,

and to my family members

for their invaluable support, love and extraordinary courage

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements of the degree of Master of Science

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May 2020

Chairman : Syafinaz Amin Nordin, MBChB, MPath, MHEd
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Continuous ambulatory peritoneal dialysis (CAPD) is a reliable and cost-effective method that offers the advantage of greater higher molecular-weight substances clearance compared to hemodialysis. However, peritonitis remains a major complication that leads to increased morbidity and mortality in CAPD patients. Although a successful diagnosis of peritonitis highly depends on the isolation of pathogens, in routine microbiology laboratory practices, identification of pathogen is being a great challenge due to high rate of culture-negative peritonitis. In order to reduce the incidence rate of culture-negative among the CAPD patients, this study was aimed to evaluate and optimize the performance of Tween 80 incorporated media at three different concentrations (0.1%, 1% and 2%). Of the 121 peritoneal fluid samples collected from CAPD patients suspected for peritonitis between September 2018 to June 2019 at Hospital Kuala Lumpur, 109 patients (90.1%) were showed to be fulfilling the criteria for peritonitis proposed by International Society for Peritoneal Dialysis (ISPD) guidelines. Among the seven different culture media used, including Tween 80 incorporated blood agar at three different concentrations, blood agar without Tween 80, MacConkey agar, Sabouraud's dextrose agar and brain heart infusion agar, Tween 80 incorporated blood agar yielded the highest positive culture (23/121) than all the other standard media. The analysis by Chi Square revealed significant difference ($p < 0.001$) between the three concentrations of Tween 80 tested in this study. Among the three concentrations optimized, Tween 80 incorporation at 0.1% has been the best concentration that support the optimum growth of all Gram-positive organisms, Gram-negative organisms and yeast cells simultaneously. The utilization of broad-range PCR using 16S rRNA typing and 5.8S rRNA typing for both bacteria and fungi identification has significantly

increased the positive rate from 15.7% by culture method to 22.3% by molecular identification. Four additional bacterial species and one additional fungi was identified by molecular method in comparison with culture method. The utilization of gene sequencing has improved the rapid identification of pathogens in culture-negative samples suspected for peritonitis. However, due to cost constraint as a major limitation in many hospitals, conventional culture method is still preferred to be the cost-effective and reliable identification method for the management of CAPD associated peritonitis. The combination of culture and molecular method has revealed that, the occurrence of Gram-positive bacterial peritonitis (69.6%) was the highest followed by Gram-negative bacterial peritonitis (21.8%) and fungal peritonitis (8.6%) in CAPD patients. Majority of the pathogen recovery by both molecular and culture methods were found to be similar in identification. Therefore, Tween 80 incorporated media has provided satisfactory result in the management of peritonitis diagnosis. In conclusion, this study suggest that the Tween 80 incorporated media at 0.1% concentration has the potential to be used as a single medium for the optimum isolation of pathogens in CAPD peritonitis. The molecular method can be used as a supplementary test to support the culture result and not as a standalone test for pathogen identification due some limitations. This approach can be applied in all diagnostic laboratories, particularly in resources-limited settings to enhance the yield of pathogens in CAPD associated peritonitis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN TERHADAP PENGGUNAAN MEDIA DENGAN TWEEN 80 UNTUK MENINGKATKAN ISOLASI PATOGEN DARIPADA CECAIR PERITONEAL DI KALANGAN PESAKIT CAPD DI SEBUAH HOSPITAL AWAM DI KUALA LUMPUR, MALAYSIA

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Continuous ambulatory peritoneal dialysis (CAPD) merupakan sejenis kaedah dialisis yang boleh dipercayai and kos efektif dengan kelebihan dalam pelupusan bahan molekul yang berat berbanding dengan hemodialisis. Walau bagaimanapun, peritonitis kekal sebagai suatu komplikasi yang utama yang menyumbang kepada peningkatan kadar morbiditi dan mortaliti di kalangan pesakit CAPD. Walaupun kejayaan diagnosis peritonitis sangat bergantung kepada isolasi, pada amalan rutin di makmal mikrobiologi, identifikasi patogen merupakan suatu cabaran yang hebat disebabkan oleh kadar kultur negatif yang tinggi. Bagi mengurangkan kadar kultur negatif di kalangan pesakit CAPD, kajian ini bertujuan untuk menilai dan mengoptimumkan penggunakan media kultur dengan Tween 80 pada tiga kepekatan (0.1%, 1% dan 2%) yang berlainan. Daripada 121 cecair peritoneum yang diperolehi daripada pesakit CAPD yang disyaki mengalami peritonitis dari September 2018 sehingga Jun 2019 di Hospital Kuala Lumpur, 109 pesakit (90.1%) telah memenuhi kriteria untuk jangkitan peritonitis berdasarkan cadangan International Society for Peritoneal Dialysis (ISPD). Di antara tujuh media kultur yang digunakan, termasuk agar darah dengan Tween 80 pada tiga kepekatan yang berlainan, agar darah tanpa Tween 80, agar MacConkey, agar Sabouraud's dextrose dan agar brain heart infusion; agar darah dengan Tween 80 menghasilkan kultur positif yang tertinggi (23/121) berbanding dengan kesemua media yang lain. Analisa data oleh *Chi Square* menunjukan perbezaan yang signifikan ($p < 0.001$) di kalangan tiga kepekatan yang diuji dalam kajian ini. Antara tiga kepekatan yang dioptimumkan, Tween 80 media pada kepekatan 0.1% didapati menyokong pertumbuhan kesemua organisma Gram-positif, Gram-negatif dan kulat secara optimum. Penggunaan keadah molekular dengan 16S rRNA and 5.8S rRNA untuk identifikasi kedua-dua bakteria and kulat telah

meningkatkan kadar positif secara signifikan daripada 15.7% melalui kaedah kultur kepada 22.3% melalui kaedah molekular. Empat spesis tambahan bakteria dan satu spesis tambahan kulat telah diperolehi melalui kaedah molekular berbanding dengan kaedah kultur. Penggunakan kaedah penjujukan gen telah menambahbaik identifikasi patogen daripada sampel kultur negatif yang disyaki peritontis. Walau bagaimanapun, disebabkan oleh kekangan kos sebagai halangan utama di kebanyakan hospital, kaedah kultur konvensional masih menjadi pilihan sebagai kaedah identifikasi yang berkos efektif dan boleh dipercayai dalam pengurusan peritonitis berkaitan CAPD. Hasil gabungan kaedah kultur dan kaedah molekular menunjukan, kejadian peritonitis disebabkan oleh organisma Gram-positif (69.6%) adalah tertinggi diikuti oleh peritonitis yang disebabkan oleh organisma Gram-negatif (21.8%) dan kulat (8.6%) di kalangan pesakit CAPD. Kebanyakan daripada hasil pemencilan patogen melalui kedua-dua kaedah molekular dan kaedah kultur menunjukan persamaan yang ketara. Oleh itu, penggunaan media dengan Tween 80 telah memberi keputusan yang memuaskan dalam pemgurusan diagnosis peritonitis. Dalam kesimpulan, kajian ini mencadangkan agar darah dengan Tween 80 pada kepekatan 0.1% berpotensi sebagai media tunggal untuk pemencilan patogen yang optimum dalam peritonitis berkaitan CAPD. Kaedah molekular boleh digunakan sebagai ujian tambahan untuk menyokong keputusan kultur, malah bukan sebagai ujian '*standalone*' untuk identifikasi patogen disebabkan oleh kekangan tertentu. Pendekatan ini boleh digunakan di semua makmal diagnostik, terutamanya di organisasi dengan sumber yang terhad untuk meningkatkan pemencilan patogen dalam peritonitis berkaitan CAPD.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ATCC	American type culture collection
BA	Blood agar
BLAST	Basic Local Alignment Search Tool
bp	Base pair
°C	Degree Celsius
CAPD	Continuous ambulatory peritoneal dialysis
CoNS	Coagulase-negative *Staphylococcus*
g	Gram
hrs	Hours
ISPD	International Society for Peritoneal Dialysis
L	Liter
ml	Millilter
MNRR	Malaysian National Renal Registry
PCR	Polymerase chain reaction
PMN	Polymorphonuclear leukocytes
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
16S rRNA	16 subunit ribosomal ribonucleic acid
sec	Seconds
spp	Species
SPSS	Statistical Package for the Social Sciences
TBE	Tris-borate-EDTA
V	Voltage
WBC	White blood cells
µg	Microgram
µL	Microliter
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Introduction

A recent study that forecasting the incidence and prevalence rate of end stage kidney disease (ESRD) in Malaysia (2017) showed that the incidence of patients with ESRD requiring dialysis has been growing rapidly at an alarming stage from 18 per million population (pmp) in 1993 to 231 pmp in 2013 (M. A. Bujang*et al*,. 2017). The 22nd Report of the Malaysian Dialysis and Transplant Registry in 2013 recorded among the total number of 32,026 patients receiving dialysis treatment, majority of them (29,192) were treated by haemodialysis (91%), while only 2,834 patients were on peritoneal dialysis (9%) (M. A. Bujang*et al*,. 2017). This trend of increasing renal failure cases in Malaysia has not only demand for the need of establishing more dialysis centers but also to improve the existing diagnostic system to ensure the right treatment to be given at the right timing.

In peritoneal dialysis, the internal lining of the abdomen acts as an artificial kidney. During the peritoneal dialysis, fluid is drained into the peritoneal cavity, allowed to sit there for several hours while it absorb the waste products and then drained them out. This process will be repeated several times in a day and because it is a continuous process, it is known as continuous ambulatory peritoneal dialysis (CAPD). CAPD represents a reliable and cost effective method which offers the advantage of greater clearance of higher molecular-weight substances compared to hemodialysis (Schweiz, 1981).

Despite the significant advance in prevention, increased morbidity of ESRD patients and antibiotic therapy during the past two decades, CAPD procedures often challenged by serious infection which leads to peritonitis (Szeto *et al*,. 2005). Peritonitis is the most common complication in CAPD patients in which an inflammation of the abdominal wall occur due to the infection of peritoneal fluid. A report documented by Malaysian National Renal Registry (MNRR) in 2011 recorded 15.6% as the annual death rate of the peritoneal dialysis patients (Lim *et al*,. 2011). Around 18% of the infected-related mortality in peritoneal dialysis patients is the result of peritonitis.

Peritonitis is usually caused by a single pathogen that originates from the normal skin flora or from upper respiratory tract (Golper*et al*,. 1996). Gram positive cocci such as *Staphylococcus epidermis*, other *CONS* and *Staphylococcus aureus* are the most frequent etiological agents of peritonitis in CAPD patients worldwide. Enterococcal peritonitis though uncommon, is also a serious complication of peritoneal dialysis. Polymicrobial peritonitis was significantly more common in *Enterococcus* species infection and it frequently

associated with catheter loss, change in dialysis modality and death (Barrett*et al*,. 2009). *Escherichia coli* and *Klebsiella* species are the most commonly occurring enteric Gram-negative rods in all episodes of peritonitis (Kim *et al*,. 2004). Some recent studies proposed that fungal peritonitis, particularly by *Candida* species are emerging to be an important cause of CAPD peritonitis especially in those immunocompromised patients with impaired or weakened immune system (Goldie *et al*,. 1996; Matsumoto *et al*,. 2002).

A good clinical microbiology laboratory support is highly essential for the diagnosis and management of CAPD patients. Non-conclusive identification of causative agents with several cases of culture-negative samples lead to non-specific antimicrobial therapy, hence contributes to the increase number of peritonitis-related mortality (Piraino*et al*,.2005). The recovery of pathogen from CAPD patients with peritonitis lacks sensitivity because the large volume (2 litres) of dialysis fluid in the peritoneal cavity dilutes the concentration of the pathogens in the small amount of peritoneal fluid sample send to the laboratory for culture and microscopy. The negative culture rate in most Malaysian hospitals, including Hospital Kuala Lumpur, as reported by the Malaysian Dialysis and Transplant Registry in 2016 was 24.5%, which is higher than the international standards.

Several studies have reported different methods to increase the sensitivity and yield of pathogens in CAPD peritonitis. The use of automated Blood Culture System (BACTEC / Bacte Alert) and concentrating large volume (50ml) of peritoneal fluid by centrifugation and culturing of the sediment onto media improves the recovery of the pathogens (Azap*et al*,. 2006; Chow *et al*,. 2007). Lysis of white blood cells (WBC) by non-ionic surfactant such as Triton X 100 and Tween 80 to release the intracellular organisms also increases the yield of pathogen from peritoneal fluid (Iyer and Kapoor, 2009).

However, prior antibiotic treatment before the sample collection has been a big challenge in the isolation of the infectious agent. Previous antibiotic treatment enables the bacteria to grow on media by decreasing the number of bacteria in the peritoneal fluid sample sends for culture purpose. Therefore, broad spectrum PCR with RNA sequencing have shown to be a complement conventional method in the management and diagnosis of CAPD patients by detecting even a small amount of bacterial DNA in the peritoneal fluid samples collected from peritonitis suspected patients (Kim *et al*,. 2012). 16s rRNA and ITS gene sequencing has been used in a recent study to identify common bacterial and fungal pathogens directly from the peritoneal fluids without culturing the samples (Ahmadi *et al*,. 2013).

A positive culture is extremely essential to assist the clinician towards the correct management of peritonitis and prevention of recurrence. Therefore, the current study is aimed to evaluate the performance of Tween 80 incorporated

media at three different concentrations to increase the yield of pathogen from peritoneal fluid samples of CAPD patients at Hospital Kuala Lumpur.

1.2 Research Hypothesis and Objective

Research Hypothesis

Surfactant containing media may increase the yield of pathogens from peritoneal fluid of CAPD patients thus increase the positivity culture rate.

General Objective

To increase the isolation rate of pathogens from peritoneal fluid samples among the CAPD patients in Hospital Kuala Lumpur.

Specific Objective

1- To evaluate the performance of Tween 80 incorporated media at different concentration to increase the positivity culture rate from peritoneal fluid samples.

2- To compare the performance of Tween 80 incorporated media with standard culture media

3- To utilize molecular isolation method (16s rRNA and 5.8s rRNA gene sequencing) for the direct detection of pathogens from peritoneal fluid of CAPD patients

4- To determine the microbiological profile of bacteria and fungi from CAPD patients with peritonitis at Hospital Kuala Lumpur.

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