Evaluation of a Chromogenic Culture Medium Versus Polymerase Chain Reaction for Diagnosis of Clostridium Difficile in Antibiotic Induced Diarrhea

Thesis Submitted for Fulfillment of M.Sc. Degree
In Clinical and Chemical Pathology

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2012

Special Thanks

Special thanks to my family who suffered a lot with me through this thesis. Thanks to my friends and all who helped me in fulfilling this thesis.

ACKNOWLEDGEMENT

Before all and after all thanks to GOD

I would like to express my deepest gratitude to ***Prof. Nada Nabil Nawar***, Professor of clinical and chemical, faculty of medicine, Cairo University for her sincere guidance, Kind supervision, continuous encouragement and valuable instructions throughout the whole work.

I am very grateful to ***Prof. Mona Mohiedden A.Haleim,*** professor of Clinical and Chemical pathology, Faculty of medicine, Cairo University for her constant assistance, valuable instructions, generous guidance and precious time she offered me throughout this work to make it complete.

Many thanks for ***Dr. Rasha Hamed Al Sherif,*** Lecturer of Clinical and Chemical pathology, Faculty of medicine, Cairo University for her beneficial help, kind assistance, constant support, and valuable supervision.

Lastly, I wish to thank all my professors, staff members and my colleagues for their kind help and support.

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List of Abbreviations

CDAD	Clostridium difficile associated diarrhea
C. Difficile	Clostridium Difficile
AAD	Antibiotic associated diarrhea
WBC	White blood cells
DM	Diabetes mellitus
GTPases	Guanosine triphosphatasis
Rho family	a family of small signaling G protein (more specific, a GTPase)
CHO cells	Carbohydrate cells
C.T.	Computerized tomography
ADP	Adenosine diphosphate
PCR	Polymerase chain reaction
ELISA	Enzyme-Linked Immunosorbent Assay
PFGE	Pulsed field gel electrophoresis
MLVA	Multilocus variable-number tandem-repeat analysis
MLST	multilocus sequence typing
IV	Intravenous
MIC	Minimal inhibitory concentration

IgM	Immunoglobulin M
IgG	Immunoglobulin G
IgA	Immunoglobulin A
tpi	Triose phosphate isomerase
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
dTTP	Deoxythymidine triphosphate
TAE	Tris acetate Edta buffer
Tcd	Toxin of clostridium difficile
HSM	Hepatosplenomegaly
S.S agar	Salmonella Shigella agar
ICUs	Intensive care units
RBCs	Red blood cells
HIV	Human immunodeficiency virus
CDI	Clostridium difficile infection
IBD	Inflammatory bowel disease

ABSTRACT

Key words: Antibiotic associated diarrhea-Clostridium Difficile-Polymerase chain reaction-Chromogenic culture media.

Background: Antibiotic associated diarrhea (AAD) can be a significant problem resulting in incomplete duration of therapy and development of microbial resistance and can cause severe complications e.g. electrolyte imbalances, dehydration, pseudomembranous colitis, toxic megacolon or even death.

Clostridium Difficile is the leading cause of antibiotic associated diarrhea in hospitalized patients.

Materials and Methods: In this work we aimed to evaluate Chromogenic agar versus polymerase chain reaction in diagnosis of Clostridium Difficile infection.

The study included 100 cases of antibiotic associated diarrhea and 20 completely healthy individuals as control group

Results: The results was that by PCR for cases, 2/100 cases were positive for toxin B,and one/100 case positive for Binary toxin, no cases were positive for toxin A, 2/100 cases were positive for tpi gene, for control group no samples were positive for any toxin or tpi gene.

By chromogenic agar, none of cases or control was positive.

Conclusion: we conclude that PCR is superior to Chromogenic agar and it is better in diagnosis of toxigenic Clostridium Difficile.

Recommendations: Further recommendations were suggested before culture as treating sample with alcohol shock in order to enrich spores and kill vegetative forms and decrease growth of flora, collecting sample and culture on the spot better than using carryblair so keeping viability of cells. Also another recommendation for PCR is the usage of positive control for Clostridium Difficile will be better.

Introduction:

Clostridium Difficile is an obligate anaerobic, spore-producing, gram-positive rod that was first described in 1935 ***(Bartlett et al., 1977)***. Its link with pseudomembranous colitis and Clostridium Difficile–associated diarrhea (CDAD) was established in 1978 ***(Poutanen and Simor, 2004)***. It is the implicated pathogen in 20% to 30% of patients with antibiotic-associated diarrhea, 50% to 75% of those with antibiotic-associated colitis, and more than 90% of those with antibiotic-associated pseudomembranous colitis ***(Kelly et al., 1994)***. CDAD is an important nosocomial infection associated with an increase in length of hospital stay and cost and substantial morbidity and mortality ***(Wilcox et al., 1998).***

Prevalence rates of C. Difficile depend on the patient population, antibiotic prescribing patterns, endemic strains, and criteria used to define antibiotic-associated diarrhea ***(Thielman and Wilson, 2005)***. Two major toxins are produced by Clostridium Difficile, an enterotoxin and a cytotoxin ***(Sun et al., 2010)***, of which the enterotoxin is thought to be the main cause of the disease symptoms ***(Wilkins, 1987)***.

Examination of faecal filtrates for cytotoxic effect neutralizable by cross reacting Clostridium Sordellii antitoxin in monolayers of various cell lines has become the 'gold standard' *test* ***(Lyras et al., 2009).***Technical difficulties in maintaining cell lines, time and cost preclude its availability in many laboratories. Enzyme immunoassays (EIAs) are commercially available for the detection of toxins ***(Barbut et al., 1993)***. Toxin degradation by proteases normally occurring in faeces decreases sensitivity with time, a particular problem if specimens have to be referred to a central laboratory ***(Brazier, 1993)***.

Non-toxigenic strains lack part or all of the genes encoding these toxins (**L*yras et al., 2009)***. While PCR could overcome the requirement for fresh specimens and provide a more sensitive test, specific for each C. difficile toxin.Moreover a Chromogenic culture medium for Clostridium Difficile has been developed to facilitate accurate diagnosis of C.Difficile ***(Perry et al., 2010).***

Aim of work

In this work we aimed to evaluate Chromogenic agar versus polymerase chain reaction in diagnosis of Clostridium Difficile infection.

Background:

In 1935, Hall and O'Toole first isolated a gram-positive, cytotoxin producing anaerobic bacterium from the stool of healthy neonates ***(Kelly et al., 2008).*** They named it Bacillus difficilis to reflect the difficulties they encountered in its isolation and culture.

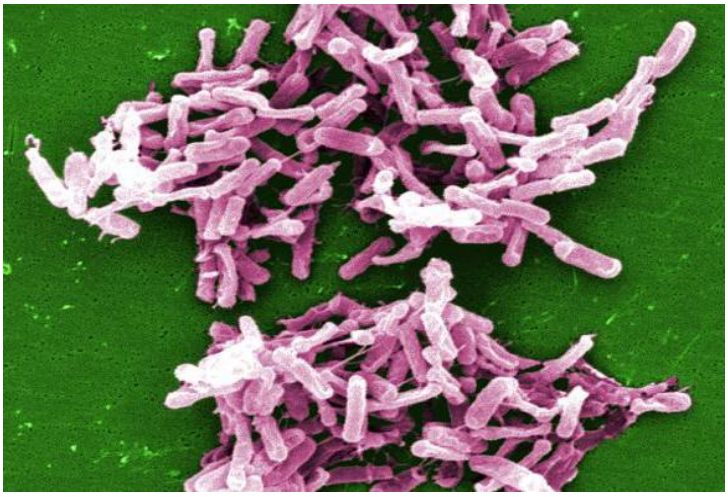

Figure (1): Micrograph of clostridium difficile *(Sebaihia et al., 2006)*.

The first report of antibiotic-associated diarrhea (AAD) was found in the Bulletin of the Johns Hopkins Hospital of 1893, where John Finney and Sir William Osler described the case of a young woman who died of a severe case of "diphtheric colitis" shortly after gastric surgery ***(Finney, 1986)*** .It was not until the mid-1900s, with the use of preoperative antibiotics, that AAD is a common medical problem.

For many years, the reason of the pseudomembranous colitis remained elusive; indeed, the term staphylococcal enterocolitis was used, reflecting the belief that the disease was commonly caused by staphylococci. In the 1970s, important observations of clindamycin-associated pseudomembranous colitis and the demonstration of the potent cytopathic effects of Clostridium Difficile–derived toxin in animal models established the cause and pathogenesis of this condition ***(Mcfarland, 1998).***

C. Difficile diarrhea refers to a wide spectrum of diarrheal illnesses caused by the potent toxins produced by this organism, including cases of severe colitis with or without the presence of pseudomembranes ***(Poutanen and Simor 2004).***

C. Difficile is the leading cause of hospital-acquired diarrhea, known as C.Difficile-associated disease, in the United States. The estimated number of cases of C.Difficile-associated disease exceeds 2million per year ***(Wilkins and Lyerly, 2003)***, with total additional health care costs approaching US$1 billion annually ***(Kyne et al., 2002).***

There are several possible explanations for the increase in C. difficile disease during the past three decades. First, better detection methods have almost certainly contributed to the increase in reported cases of C. Difficile-associated disease. Second, the high-frequency use of antibiotics and chemotherapeutics increases the likelihood of acquiring C.Difficile-associated disease. Third, as the frequency of disease has increased, hospitals have become contaminated with spores of C.Difficile, making infection of susceptible patients more probable ***(Wilkins and Lyerly, 2003).***

The C. Difficile bacterium has two forms, an active infectious form that cannot survive in the environment for prolonged periods, and a nonactive, "noninfectious" form a spore that can survive in the environment for prolonged periods. Spores cannot cause infection directly, when they are ingested but they transform into the active infectious form ***(Akerlund et al., 2008).***

C.Difficile spores are found frequently in hospitals, nursing homes, extended care facilities, nurseries for newborn infants. They can be found on Bedpans, Furniture, Toilet seats, linens, Telephones, Stethoscopes,

Fingernails, Rings (jewelry),Floors, rooms, and Diaper pails. They even can be carried by hands of care givers and by pets. Thus, these environments are a ready source for infection with C.Difficile. ***(Akerlund et al., 2008).***

Epidemiology:

Each year, C. Difficile infection results in about 3 million cases of diarrhea and colitis in the United States. The case mortality rate is approximately 1 to 2.5 percent. C. Difficile infection was thought to result from an overgrowth of commensal organisms in the colon; however, studies have shown that fewer than 3 percent of adults carry this pathogen ***(Hurley and Nguyen, 2002).***

C.Difficile occurs primarily in the hospital setting, where the organism has been cultured from beds, floors, windows, and toilets, as well as the hands of hospital workers who provide care for patients with C. Difficile infection. The organism can persist in hospital rooms for 40 days after infected patients have been discharged ***(Hurley and Nguyen, 2002).***Patients re-admitted after recent hospitalizations are found to have a high prevalence of C. Difficile colonization, representing an important source of infection. Because of the sporulating properties of this organism, all these observations have suggested an important role for cross-contamination between patients. ***(Kim et al., 1981)***.

During the past few years, there has been renewed interest in C. difficile diarrhea reflecting a form of disease that is more frequent, more severe, and more refractory to standard treatment. These epidemiologic changes appeared a new hypervirulent, epidemic strain of C.Difficile, referred to as BI/NAP1/027, which produces higher concentrations of toxins A and B and produces binary toxin that is of uncertain significance, and more resistant to treatment e.g. fluoroquinolones and appeared to have short deletion in (Tcd C gene) functions as a negative regulator of toxins ***(Geric et al., 2006).***

C.Difficile infection predominantly affects elderly, hospitals and nursing home patients ***(McDonald et al., 2006)***. However, Centers for Disease Control and Prevention warns about infection in populations not previously considered at risk ***(Kuijper and van Dissel, 2008)***, these population include young and previously healthy persons who were not exposed to a hospital or health care environment or antimicrobial therapy. Close contact with patients who have C.Difficile infection was the only evident risk factor in some cases, indicating the importance of direct person-to-person spread ***(Nolan et al., 1987)***.

Severe infection leading to colectomy and then death was also described in young women in the peripartum period ***(Lefebvre et al., 2006)***. This increased awareness of the possibility of fulminant C. Difficile infection in atypical settings should facilitate diagnosis and treatment earlier.

Prevalence:

The occurrence of antibiotic associated diarrhea varies greatly and is influenced by many factors, as nosocomial outbreaks, patterns of antimicrobial use, and individual susceptibility. It is estimated that 10% to 15% of all hospitalized patients treated with antibiotics will develop AAD and many of hospitalized patients will become asymptomatic carriers. Infection rates for C. Difficile are reported to be around 10% after 2 weeks of hospitalization, but may reach 50% after 4 or more weeks ***(Surawicz and McFarland, 1999).***

Prevalance of C.Difficile in symptomatic patients has been examined frequently in hospital settings. In one hospital 30% of adult patients who developed diarrhea during hospitalization were found to have this organism ***(Gerding et al., 1986).***