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

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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.

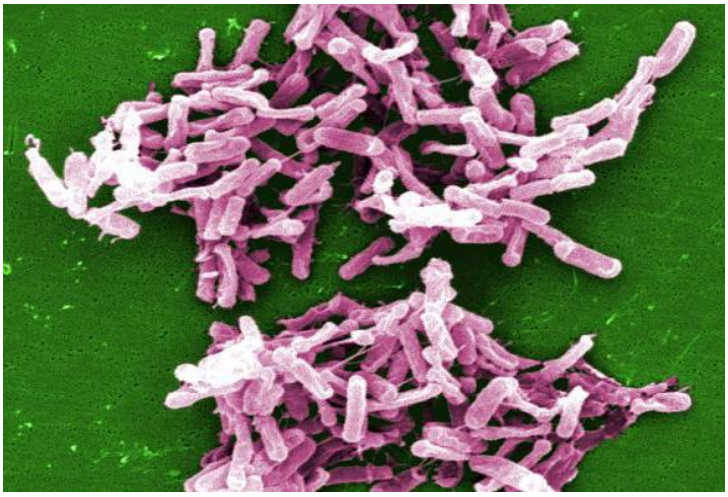

Figure (1): Micrograph of clostridium difficile *(Sebaihia et al., 2006)*.

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).***