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NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

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MEDICAL AND CLINICAL MICROBIOLOGY PROGRAM

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MASTER OF SCIENCE THESIS

THESIS SUPERVISOR

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NICOSIA

2020

The Directorate of Graduate School of Health Sciences,

This study has been accepted by the thesis committee in Medical and clinical microbiology program as a Master of Science Thesis.

Thesis committee:

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Approval:

According to the relevant article of the Near East University Postgraduate Study-Education and Examination Regulation, this thesis has been approved by the above-mentioned members of the thesis committee and the decision of the board of Directors of the institute.

Prof. K. Hüsnü Can BAŞER, PhD
Director of Graduate School of Health Sciences

DECLARATION

I hereby declare that this thesis work entitled: “PREVALENCE OF *Clostridium difficile* A-B toxins ASSOCIATED DIARRHEA IN NEAR EAST UNIVERSITY HOSPITAL

” is the product of my own research work undertaken under the supervision of Assoc. Prof. Kaya SÜER. No part of this thesis was previously presented for another degree or diploma in any University elsewhere, and all information in this document has been obtained and presented in accordance with academic ethical conduct and rules. All materials and results that are not original to this work have been duly referenced.

Signature: Ahmed Nouri Alsharksi

Date: 5/6/2020

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ABSTRACT

Background: Globally, Nosocomial diarrhea is given due attention as a result of its prevalence and emergence outbreak in some regions. Morbidity and mortality rate of this anaerobic bacteria account for about 500,000 cases annually in the United States North America, Europe, and Asia seems to have a consistence rise in prevalence rate of C. difficile. Little data exist on the prevalence of hospital related C. difficile in Turkey and North Cyprus. This retrospective study focuses on investigating the rate of C. difficile in Near East University Hospital.

Materials and methods: Records of patients admitted to the different units of the university hospital were obtained for the period of 1st September 2015 to 31st December 2018. A total of 230 sample data were used and analyzed using SPSS. Data variables used were gender, age, department and patient admittance category (in-patient and out-patient). Ages of the patients were classified into two categories.

Results: gender category shows no statistically significant between male and Female (p=0.822). Higher rate of *C.difficile* positive (18.2%) was found among age group 20-44 years, and maintain constant prevalence of 15.5% for the age group 45 years and above. But there was no statistical significant differences in age group as chi-square result gives a p value =0.721. A statistically significance was found in the patient status (inpatient and outpatient) as p value was less than alpha (p=0.018).

Conclusion: 9.70% positive rate was found from this study from the in-patient's record while 21.30% positive were from the outpatients. This is due to the unregulated guideline in use of antibiotics by outpatients obtained from pharmaceutical shops. This study suggests a reverse of strict guidelines in the use of antibiotics within North Cyprus by hospitals and Pharmacies.

Keywords: Prevalence, Clostridium difficile, Toxins, Near East University, TRNC

ÖZET

Amaç: Küresel olarak, nosokomiyal diyare, bazı bölgelerde sık görülmesi ve salgınlara yol açması nedeni ile dikkat edilmesi gereken bir durumdur. Bu anaerobik bakterilerin morbidite ve mortalite oranı, Amerika Birleşik Devletleri Kuzey Amerika, Avrupa ve Asya'da yılda yaklaşık 500.000 vakayı oluşturmaktadır. Türkiye ve Kuzey Kıbrıs'ta hastaneye bağlı C. difficile prevalansı hakkında çok az veri bulunmaktadır. Bu retrospektif çalışma Yakın Doğu Üniversitesi Hastanesi'nde C. difficile oranının araştırılmasına odaklanmaktadır.

Gereç ve yöntem: 1 Eylül 2015 - 31 Aralık 2018 tarihleri arasında üniversite hastanesinin farklı birimlerine başvuran hastaların kayıtları alındı. Kullanılan veri değişkenleri cinsiyet, yaş, bölüm, yatan ve ayaktan olarak kategorize edildi. Hastaların yaşı iki kategoriye ayrıldı. Toplam 230 numune verisi kullanıldı ve SPSS kullanılarak analiz edildi

Bulgular: cinsiyet kategorisi, erkek ve kadın arasında istatistiksel olarak anlamlı bulunmadı (p = 0.822). 20-44 yaş grubunda daha yüksek C.difficile pozitif (% 18,2) oranı saptandı ve 45 yaş ve üstü yaş grubu için% 15,5'lik sabit yaygınlığı korudu. Ancak ki-kare sonucu p değeri = 0.721 verdiği için yaş grubunda istatistiksel olarak anlamlı bir fark yoktu. Hasta statüsünde (yatarak ve ayakta tedavi gören hasta) p değeri alfadan daha düşük olduğu için istatistiksel olarak anlamlı bulundu (p = 0.018

Sonuç: Bu çalışmada yatan hasta grubunda % 9,70 oranında ve ayaktan hasta grubunda % 21,30 oranında C.difficile toksin A-B pozitifliği bulunmuştur. Bunun nedeni, ülkede reçetesiz antibiyotik satışını engelleyen bir kanun olmakla beraber, denetim mekanizmasındaki eksiklikler nedeni ile yaygın antibiyotik kullanımıdır. Bu çalışma, Kuzey

Kıbrıs'ta hastaneler ve Eczaneler tarafından antibiyotik kullanımıyla ilgili katı kuralların yetersizliğini göstermektedir.

Anahtar Kelimeler: Prevalans, Clostridium difficile toksin A/B, Yakın Doğu Üniversitesi, KKTC

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LIST OF ABBERVIATIONS

CCA: Cell-based cytotoxic assay

CDAD: Clostridium difficile associated disease

CD: *Clostridium difficile* (CD)

CDI: Clostridium difficile infection

PCR: Polymerase chain reaction

US CDC: United States Centre for Disease Control and Prevention

FDA: Food And Drug Administration

GTD: glucosyltransferase domain

CPD: cysteine protease domain

DD: delivery domain

RBD: receptor binding domain

PPIs : proton pump inhibitors

GDH : glutamate dehydrogenase

IBDs: inflammatory bowel diseases

SLPs: Surface layer proteins

FMT: Faecal microbiota transplantation

PBP: penicillin-binding protein:

OD: Optical Density

CHAPTER ONE

INTRODUCTION

Infectious diseases account for approximately 50% of mortality cases in the tropical countries with malaria, diarrheal diseases and respiratory tract infections as the most common examples (Nawab *et al*., 2018). These diseases maybe caused by different agent such as bacteria, viruses, fungi or parasite initiating different types of symptoms from mild, acute, to severe cases. *Clostridium difficile* (CD) is a pathogen that causes alterations in the homeostasis of the intestine that lead to diarrhea due to use of antibiotics in treatment. Also, this gram-positive bacterium is a major cause of the hospital-related inflammation of the colon lining (colitis) (Vaishnavi, 2010). United States Centre for Disease Control and Prevention (US CDC 2013) declared *C. difficile* infection as a threat to public health due to bacterial drug-resistance *C. difficile* infection (CDI) is mostly associated with health-care, but some studies outside healthcare facilities was also given attention due to out-patient usage of antibiotics. Both the nosocomial and community related diarrhea tends to pose a public health concern. The Community-related case of *C. difficile* is usually identified by other method but not the normal infection control or conventional surveillance method (Tan *et al.*, 2014).

Globally, Nosocomial diarrhea is given due attention as a result of its prevalence and emergence outbreak in some regions. Severity of this disease tends to rise in some locations due to lack of early diagnosis and preventive measures. Some risk factors such as chemotherapeutic agents are associated with *C. difficile* diarrhea, usually now called *C. difficile*-associated diarrhea (CDAD) (Ergen *et al.*, 2009).

From studies, prevalence of toxigenic *C. difficile* varies among the Asian populations. In 2015, Cheng *et al*. (2015) reported the prevalence of *C. difficile* to be 19.2% in China using PCR-based technique on stool culture, while Thailand has 9.2% (Putsathit *et al*., 2017). Most of the studies use direct detection method and show a prevalence of 10.9% in India (Vaishnavi *et al*., 2015) and 9.6% in Singapore (Tan *et al.*, 2014).

Despite all these studies, hypervirulent strains tends to be low in some geographical locations and high in others.

1.1 Statement of problem

Persistence rise of CDI is observed globally in developing countries due to lack of proper and early diagnostic measures. There is a scarce study for the prevalence of *C. difficile* in Turkey and TRNC. Therefore, this study intends to investigate the prevalence of this infection in NEU hospital to start at a narrow view.

1.2 Aims and objectives of the study

The research analysis will help to determine whether the level of CDI among patients in the Near East University is increasing or decreasing so as to put a preventive measure.

To check if there is a particular period in which the infection is reported as outbreak within a particular department or multiple departments at the same period.

Also, is to check if the prevalence of *C. difficile* is more profound in in-patient or out-patient.

1.3 Scope and limitations of the research

This study is strictly based on the reported cases of *C. Difficile* A and B toxin associated with diarrhea recorded at the Near East University Hospital for certain period of time, with limited sample coverage of 230 from patients recorded by the record unit of the hospital.

CHAPTER TWO

2.0 LITERATURE REVIEW

C. difficile is nosocomial pathogenic bacteria that releases proinflammatory cytotoxins namely Toxin A and B that cause the common known *C. difficile* infection. Morbidity and mortality rate of this anaerobic bacteria account for about 500,000 cases annually in the United States (Lessa *et al*., 2015). Other members of this bacteria include *Clostridium sordellii and Clostridium novyi* (Pruitt *et al*., 2012). In the UK, patients aged 65 and above are diagnosed for the presence of CDAD without suspecting any risk factor so as to lower its prevalence (Barbut *et al*., 2003; Planche *et al*., 2008). There are two major types of toxins (A and B) that are produced and they exert different effects.

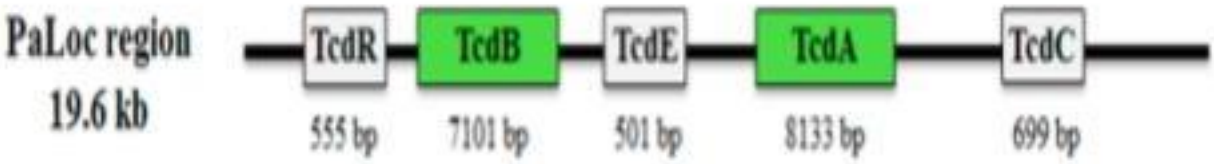
Toxin A induces the production of neurokinins and cytokines which serve a pivotal role in the pathogenesis of *C. difficile* infections. After its binding to the receptor, toxin B binds and initiates different responses that include destabilization of nucleic acid and protein levels, potassium level and structure of actin among others (Vaishnavi 2010).

Toxin B is usually targeted by the popularly approved FDA drug "Bezlotoxumab" which comprises IGHV5-51 and IGKV3-20 (Orth et al., 2014). The IGHV5-51 and IGKV3-20 are L-chain antibodies that are used in *C. difficile*. TcdB has variety of receptors that are widely spread such as chondroitin sulphate proteoglycan 4, which as both as TcdB receptor at cellular level in a knock down screening of short hairpin RNA and as a functional receptor in HeLa and HT-29 cells (Yuan *et* al., 2015). Tao *et al* (2017) reported that Wnt receptor frizzled family are also receptors of TcdB. They bind to cysteine-rich domain that is conserved on the Wnt binding site.

Most common symptoms of *C. difficile* A-B toxins include watery diarrhea which is associated with abdominal cramps. Other symptoms that occur at severe cases include colonic bleeding, high fever, and dehydration among others (Vaishnavi 2010). tcdA and tcdB genes are found on a locus known as the locus of pathogenicity (PaLoc). The PaLoc consist of five types of genes, *tcdA, tcdB, tcdC, tcdD* and *tcdE* which potentiate either a positive or negative regulatory effect (Rupnik *et al*., 2003). *TcdA* and *TcdB* has four domains namely: glucosyltransferase domain, cysteine protease domain, delivery domain, and receptor binding domain as depicted in figure 1a and b). They bind and inactivate Rho GTPase by adding glucose moiety (Pruitt et al., 2012) in a reaction known as glycosylation (Leslie *et al.,* 2015; Tam *et al.*, 2015). This modification causes perturbation of epithelial cells and paracellular flow of fluids that causes cell death due to the loss in architecture of the cells (Fig2). Ergen *et al* (2009) report the first English work on the prevalence of CDAD and nosocomial diarrhea in a Turkish university hospital.

Aside toxins A and B, PCR ribotype 027 and 028 produces a binary toxin (ADP-ribosylating toxin) (Gerding *et al* 2014). The binary toxin (CDT) consist of catalytic (CDTa) and a binding (CDTb) domain that has amino acid peptides used as signals (Rupnik et al 2003; Perelle *et al* 1997). It causes a protuberance that is important in the binding of *C. difficile* at the cell surface of the intestine. Binary toxin was first described from CD196 strain that was isolated from pseudomembranous colitis patient showing different symptoms between the negative and the positive (Popoff *et al* 1988). Few studies (Barbut *et al* 2005; Bacci *et al* 2011; Stewart *et al* 2013) suggest the link between binary toxin and austerity of CDI in negative and positive strains of TcdA and TcdB.

A



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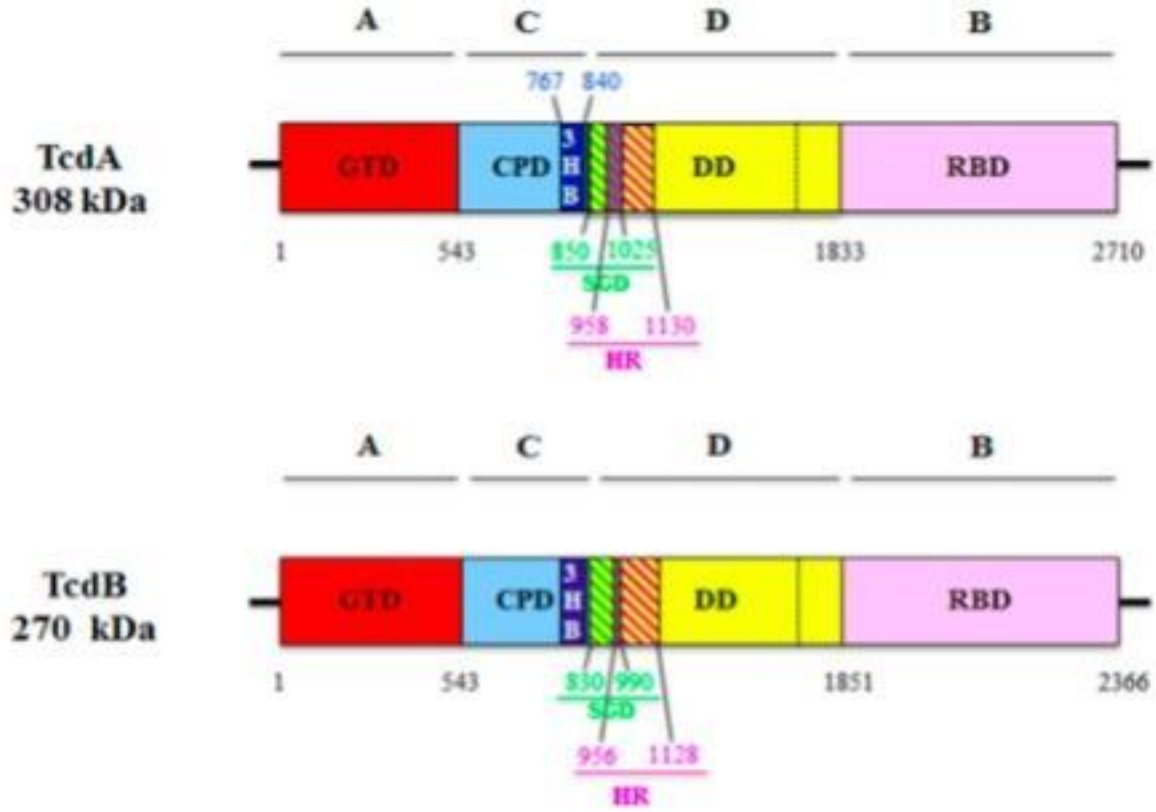

Figure 1: Schematic presentation of the PaLoc and ABCD model of toxin A and B

Source: Di Bella *et al* (2016).

GTD= glucosyltransferase domain, CPD= cysteine protease domain, DD= delivery domain, RBD= receptor binding domain.

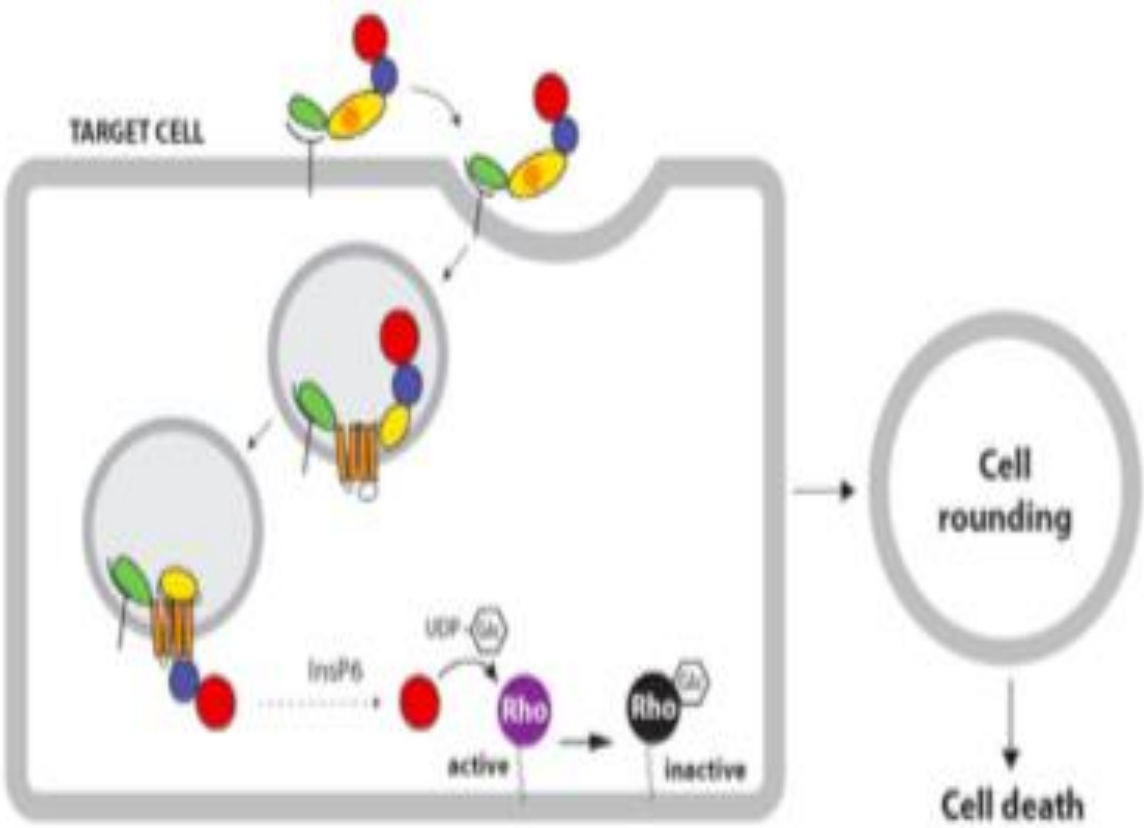

Figure 2: Schematic representation of TcdA and TcdB mechanism of intoxication that lead to cellular death.

Source: Tam *et al.* (2015).

In children, CDI tends to be somehow difficult to differentiate from gastrointestinal infections due to unspecific symptoms. But Chang *et al* (2018) reported comparable parameters such as age and proton pump inhibitors (PPIs) to be risk factors of CDI in children.

Early studies focus on healthcare related *C. difficile* which is as a result of profound findings from studies and the definition of the infection. But recently, several studies took the task of assessing the source of the infection. Some studies found out a high number of outpatients to be *C. difficile* positive, which may not be due to the use of antibiotics. Although the disease can be transmitted via contact or food production.

Community-related *C. difficile* account for about 25% of the reported cases of the infections in regions like Australia (Bloomfield and Riley 2016). Its incidence was at rise in Tel Aviv Sourasky Medical Centre from a study conducted by Na'amnih *et al*. (2017) and contact with recently hospitalized patients has been highlighted as a risk factor. This study aside mentioning sources of community associated *C. difficile* such as the environment itself, water and food chain; further suggest investigating places like animal house and humans as potential reservoirs for the infection.

Diversification in the genetic composition of the *C. difficile* play role in the transmission of the infection (Knight *et al*., 2016) in both healthcare and community. This variation was reported by Eyre *et al* (2013) in a whole genome sequence study to be >10 single nucleotide variants, and is community related. Avbersek *et al* (2009) reported the diversity of *C. difficile* among animals in Slovenia, which indicate diversity in genotypes from different

geographical regions. The study focuses on pigs, calves and horses in different farms in Slovenia. Sample isolates were tested for different strains on the basis of assigned criteria. There are 5 biological group of *C. difficile* classified as clade 1-5 which differ between regions (Knight *et al* 2015). These are as stated: Clade-1 Europe, Clade-2 North America, Clade-3 Potentially Africa, Clade-4 Asia, and Clade-5 Australia. Among the most prevalent strains of *C. difficile* in Australia is the RT 014 which is reported to account for approximately 25% of the recorded reported cases of CDI (Collins *et* al 2017). *C. difficile* lineage has varied and different types of locus. The ribotype 012 has the accessory gene regulator 1 (*agr*1) locus, ribotypes 017 and 027 has the *agr*2 locus, while *agr*3 is found in ribotypes 078 and 027 also (Knight *et al*., 2017).

Recently, the epidemiology of this infection varies substantially with respect to geographical location. Different virulence strains are found to be pronounced in regions such as Asia, ribotype 017 (Collins et al., 2013); North America, NAP1/BI/ribotype 027 (Cartman et al 2010) and ribotype 078 in Europe (Goorhuis *et al*., 2008).

2.1 *C. difficile* life cycle

The life cycle of *C. difficile* depends on distortion of the gut microbiota mostly by antibiotics to gain access and initiate its' function. As an anaerobic bacterium, it forms spores that are resistant to many environmental factors such as oxygen, disinfectants, ethanol and so on (Isidro et al 2017). As a first step of its action, spores of *C. difficile* are ingested and bind so as to pass the gastric barrier (fig 1). *C. difficile* after exiting the vegetative growth cycle

enters the sporulation via phosphorylation and produce endospores that are metabolically dormant.

Spores return to the initial vegetative cycle in order to initiate the disease in a process known as "germination" (Dembek *et al*., 2013). Spores formation in *C. difficile* are facilitated in the medium by lysozyme or bile salts (Wren 2010). Once in the intestine, the ratio of cholate and chenodeoxycholeic acid increases and it help the germination of the spores. Cholic acid help in the germination progression while chenodeoxycholate inhibit germination (Sorg and Sonenshein 2008; 2009). These acids are highly regulated by bile salt hydrolase to ensure their conversion to respective forms. Other co-germinants such as histidine and glycine were previously reported (Wheeldon *et al*, 2008; 2011). The process of germination takes about 2 hours on which it forms a vegetative cell that leads to sporulation and cytotoxic activity simultaneously in the large intestine (Koenigsknecht *et al* 2015; Dembek *et al* 2013). The two cytotoxins disrupt the epithelial cells and causes diarrhea, a major symptom of the infection. The spores spread to affect neighboring cells to cause infection.

2.2 Strains of *C. difficile*

C. difficile contain two types of strains namely flagellated and the non-flagellated, with the flagella containing flagellin (flic) which the *fliC gene* serves as an important marker for CDI (Tasteyre *et al* 2001; Vaishnavi *et al* 2015). *tcdA* and *tcdB* are the genes that encode for *C. difficile* toxin A and B respectively. Also, they cooperate together to encode for the binary toxin (Rupnik et al 2003). Toxigenic strain of *c. difficile* is usually isolated from patients with reported cancer cases, gastrointestinal disorders (GID) and patient who undergo surgery (Vaishnavi et al 2015). Most pathogenic strains have the *tcdA* and *tcdB* genes that play

different roles observed from studies (Pruitt *et al*., 2012). The clonal strain of this infection is the most predominating form found in the health-care (Singh *et al* 2015).

2.2.1 The variant Strains of *C. difficile*

C. difficile strains produce large amount of glutamate dehydrogenase (GDH) that is detected in feces during diagnostic (Cheng *et al*., 2015). Studies suggest that this method of detecting *C. difficile* is a mere preliminary method as it only indicate the presence of the bacteria but not the type of toxin it produces (Cheng *et al* 2011). Another method use is the reference method, which takes 2-3 days for completion and is based on cytotoxicity of the stool culture due to the presence of toxin A and B (Planche *et al* 2008; Poutanen and Simor, 2004).

C. difficile strains can be classified into 31 variant strains or toxinotypes on the basis insertions, deletions, and sequence mutations using toxinotype 0 as the reference strain (Eckert *et al* 2014). Despite containing the pathogenicity locus, toxinotype XI is the only type that does not produce toxins A and B (Stare and Rupnik 2010) which may be due to the characteristics of this toxinotype that differ from others.

Hypervirulent strain of CDI (toxinotype III) has 18 base pair (bp) and a deletion at position 117 of its regulatory gene *tcdC*, and accumulate the genes of toxin A and B and the binary toxin (Goorhuis *et al*., 2008). The study further revealed that the hypervirulent type 078 affect younger population than the aged, and is more of community-related infection than health-care.

PCR ribotype 027 was first reported outbreak from Canada where it mostly affects people from the Quebec. Its infection in 16 European countries was reported by the European Centre

for Disease Prevention and Control (European Centre for Disease Prevention and Control, 2008)

Ergen et al 2009 reported *C. difficile* to be the causative agent of nosocomial diarrhea in patients using toxin test and culture media.

2.3 *C. difficile* and diseases

There is existence knowledge on the concern for association of CDAD with diseases especially the metabolic diseases. *C. difficile* is found to be associated with inflammatory bowel disease, gastrointestinal disorders, solid organ transplantation, and nephritic failure to a lesser extent.

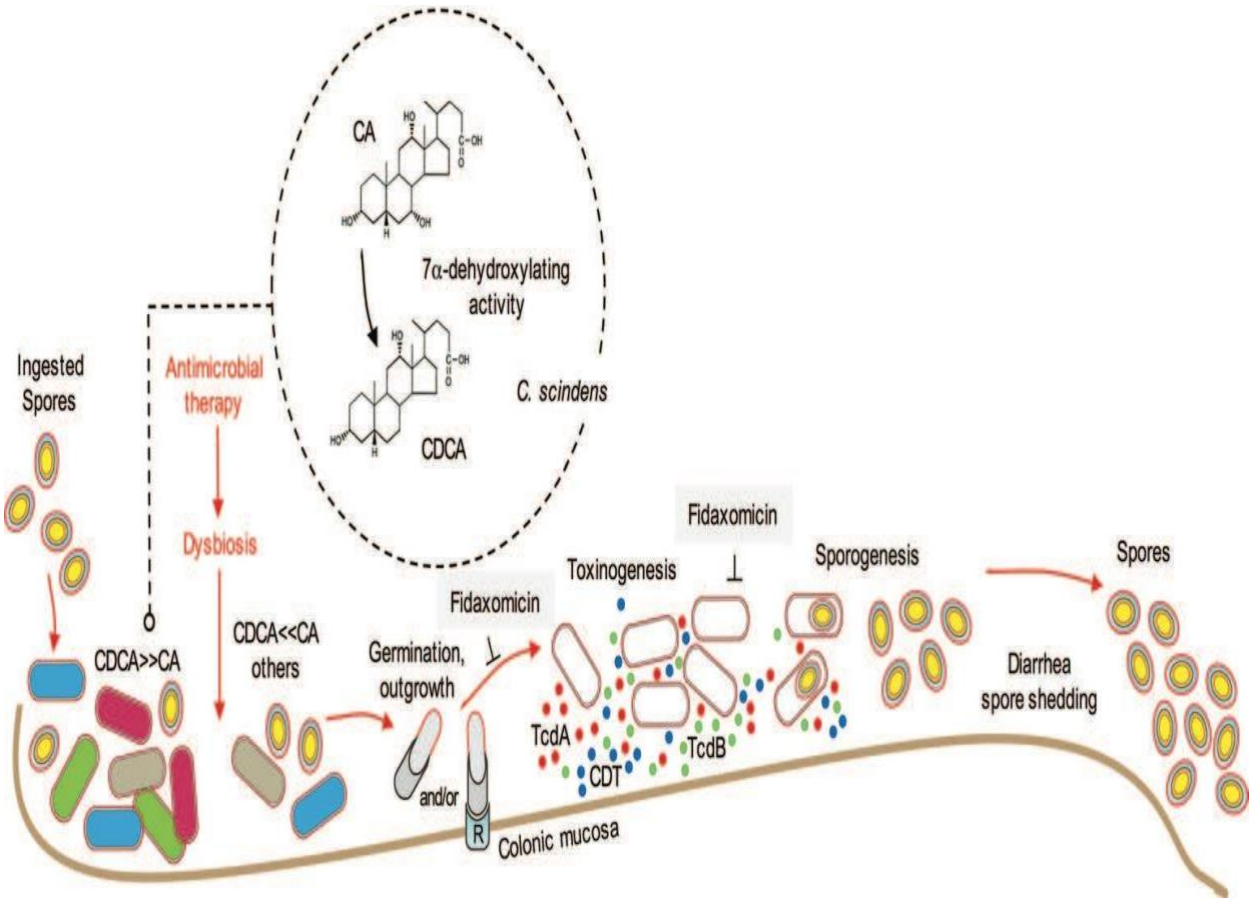

Figure 3: *C. difficile* infectious life cycle.

Source: Isidro et al (2017).

CA= cholic acid, CDCA= chenodeoxycholeic acid

2.3.1 Inflammatory bowel disease

A clear indication of rise in risk factor between community associated *C. difficile* infection and inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis, has been reported by Na'amnih *et al* (2017) in a study conducted at Tel Aviv Sourasky Medical Centre. Symptoms of CDI and IBD are similar and hardly differentiable, but the treatment approach determines the specific as CDI management involves antibiotic therapy and immunosuppression reduction while IBD involves enhancement of immunosuppression (Ananthakrishnan and Binion, 2010). This association of IBD and CDI may be due to factors such as host immunity, use of corticosteroids, and immunosuppressive medication (Issa *et al*., 2007; Rodemann *et al*., 2007).

2.3.2 Gastrointestinal

Benson *et al* (2007) reported CDAD as a risk factor for gastrointestinal disorders, which they suggest a correlation between the pathology of the two diseases. In the same study, they found out that new strains of *C. difficile* are at a rise in the study area. Recently, a multianalyte test for gastrointestinal pathogens showed enhancement of the diagnosis for suspected CD patients and has a higher sensitivity for toxin A and B (Krutova *et al*., 2019).

2.3.3 Nephrotoxicity

Different studies (Li *et al* 2018; Spinner et al 2018) investigate the possible role of CDAD as a risk factor among kidney transplant patients. None of these studies pinpoint a risk factor associated with total renal failure. But Pai *et al* (2012); Cimolai 2019 and Chang *et al* 2018 all reported a rise in serum creatinine level in patients diagnosed with CDAD. About two decades ago, Schmidt *et al* (1996) reported the cytotoxic effect of Toxin B to human embryonic kidney cells.

2.3.4 Solid organ transplantation

Solid tumors can be described as accumulated mass of tissues that does not contain liquid portion which can be benign or malignant (Gavhane *et* al., 2011). Prevalence of CDI in solid tumor patients is more pronounced than those of the hospital based on the organ transplanted (Nelson *et al*., 2018). This may be due to the long stay in hospital. Nelson *et al*. (2018) also reported that lung transplants patients are at higher risk of CDI.

2.4 Methods for CD detection

Different laboratory methods are employed for detection of CD, and these methods affect the results obtained from studies (Putsathit et al 2016). Some of the methods used in diagnosing *C. difficile* have some setbacks.

The most common methods for the detection of *C. difficile* are categorized into 5 groups (Cheng *et al*., 2011):

1. Toxigenic culture: this method is used to determine the toxigenic status of *C. difficile* isolates. It is used for epidemiological typing study.
2. Cell-based cytotoxic assay (CCA): it detects stool cytotoxic activity. It is specific and sensitive but is difficult and slow.
3. PCR-based assay: conserved gene targets that are within the pathogenicity locus of *C. difficile* are detected using this method. This test is sensitive and specific but expensive. Other drawbacks of this method include detection of *C. difficile* in healthy individuals (Vaishnavi 2010 C17).
4. Glutamate dehydrogenase assay (GDH): this assay detects a common enzyme found in *C. difficile* infection. It is sensitive but less specific

5. Enzyme Immunoassays (EIAs): it detects toxins A and/or B. Assay kits are usually used in this method. It is more specific than the GDH assay method.

Nucleic acid amplification assay is also one of the methods used in detection of *C. difficile*. Surface layer proteins (SLPs) that are encoded by the *slpA* gene serve as a marker to differentiate isolates of *C. difficile*. Both *slpA* and *fliC* genes are easily expanded by the PCR technique (Vaishnavi et al 2015). Most of the methods are based on a combination approach of different diagnostic methods to achieve an efficient result. PCR-based methods are the most effective and specific methods that are widely used in most of the developed countries (Vaishnavi 2010)

2.4.1 Storage of faecal samples for diagnosis

The need to preserve samples prior to diagnosis is a well-known phenomenon in laboratories so as to preserve the texture and properties of that sample. Faecal samples are usually stored at cold temperature, usually 4^0 C or lower temperature. This is to preserve the enzymes found in *C. difficile* that are detectable using the enzyme immunoassay method. Most studies report decline in stability *C. difficile* after long storage but the studies mostly use little samples.

Contrary to the report of Centre for Disease Control which states that *C. difficile* toxins become undetectable after 2 hours of un-refrigeration; Modi *et al* (2010 C36) reported and extension time (13 hrs.) for detection of *C. difficile* toxins from unrefrigerated human faecal samples. They also found out that the yield between samples tested within 2 h and those tested later show no significance difference.

CDI reoccur sometimes after treatment, and this phenomenon is known as recurrent CDI. The behavior of recurrent CDI is different, therefore there is a need to develop different strategies for its treatment. Adaptive immunity protects both recurrent and acute CDI, but

the gut microbiota solely help in protection without associating with adaptive immunity (Leslie et al 2019 C21). Binary toxin has been reported by Stewart *et al* (2013 C33) to be an independent predicting factor for recurrent CDI. Faecal microbiota transplantation, FMT, is among the standard therapy used for treatment of recurrent CDI using (Lee et al 2016 C48). Some studies use fresh or frozen, while others use lyophilized FMT. Gut microbiota provide protection from recurrent CDI via faecal transplantation from fresh or frozen (same donor) faecal (Jiang et al 2017 C47). The study also reports low efficacy of lyophilized product when compared with fresh product of the same donor. Lee *et* al (2016) reported that there is no difference between fresh product and frozen product during FMT.

Schwan *et al* (2009) reported a second function of CDT not only as an actin modulator but as a vehicle that helps the adherence of bacteria by creating a microtubule network that is modified at periphery of the cytoplasm.

2.5 Resistance

Drug resistance is a phenomenon that results when a drug becomes tolerant to pharmaceutical treatments. Antimicrobial resistance helps in the spread of diseases. There is a constant change in transmission of *C. difficile* due to constant change of drugs that are used to combat the infection and the rate of resistance varies among different ribotypes and regions (Isidro *et al* 2017). Bacterial resistance can be intrinsically or acquired resistant. Bacterial antimicrobial resistance is usually due to mutation in certain genes. Therefore, this makes intrinsic resistance the major cause of antimicrobial resistance with different targets in *C. difficile*. The mechanism of the resistance can be grouped into 3 (Blair *et al* 2015):

(a) Alteration of the antibiotic target

(b) Inhibition of the antibiotic

(c) Regulation/reduction of the cellular antibiotics.

In the intrinsic mechanism of resistance (fig4) antibiotic A enters the cell via the porin protein to its target site. Once in the target site, it inhibits the synthesis of peptidoglycan. Antibiotic B and C also have the same mechanism for targeting penicillin-binding protein (PBP) except that antibiotics cannot access the PBP due to its inability to cross the outer membrane. Once any antibiotic enters the cell, it is removed by efflux when the second antibiotic initiates its action via the efflux pump. This mechanism is similar for most antibiotics.

Consistent use of drugs leads to bacterial resistance by pathogens due to adaptation. There was a persistent rise in spread of 3 ribotypes (RT054, RT017, RT244) of *C. difficile* in Australia since the period of 2010 which may be as a result of different mechanisms especially due to travelers' movement in and out of Australia (Collins *et al*., 2017).

Antibiogram profile of *C. difficile* is performed from culture media so as to check its antimicrobial resistance to some common drugs used in the treatment of the infection. Pharmacodynamics, pharmacokinetics, potency and low cost, along with little effect of metronidazole render its wide use among other CDI approved drugs (Singh et al 2015). The study further reported high resistance of *C. difficile* to some common antibiotics ciprofloxacin (33.9%) and clindamycin (52.9%) with low resistance (1.3%) to metronidazole and non to vancomycin.

2.6 Treatment

Most studies tend to look for molecules that will block the pathogenesis of this infection. Tam *et al* (2015) report the first suggestion on using small molecules as *C. difficile* inhibitors

which inhibit the activity of the enzyme glucosyltransferase via non-competitive inhibition. They also confirmed the role of phloretin as an inhibitor of A-B toxin. The form of inhibition is also noncompetitive. Phloretin and methyl cholate discovered in the study were able to protect cellular damage from toxins. Probiotics are used to counter the effect of antibiotics in the management of CDAD. But the use of probiotics should also be given careful considerations due to its association with invasive diseases as reported by Enache-Angoulvant and Hennequin (2005).

Metronidazole and vancomycin are popularly known for the treatment of CDI especially severe conditions. Based on the current guideline, metronidazole hydrochloride has been recommended as a first line of defense for the treatment of severe CDI cases, but vancomycin was recently reported to be more effective than metronidazole (Stevens et al., 2017) . This study further justified the existing fear of vancomycin resistance as a result of concurrent use. Igarashi et al (2018) also reported a similar result from a meta-analysis study showing the superiority of vancomycin over metronidazole in severe CDI conditions.

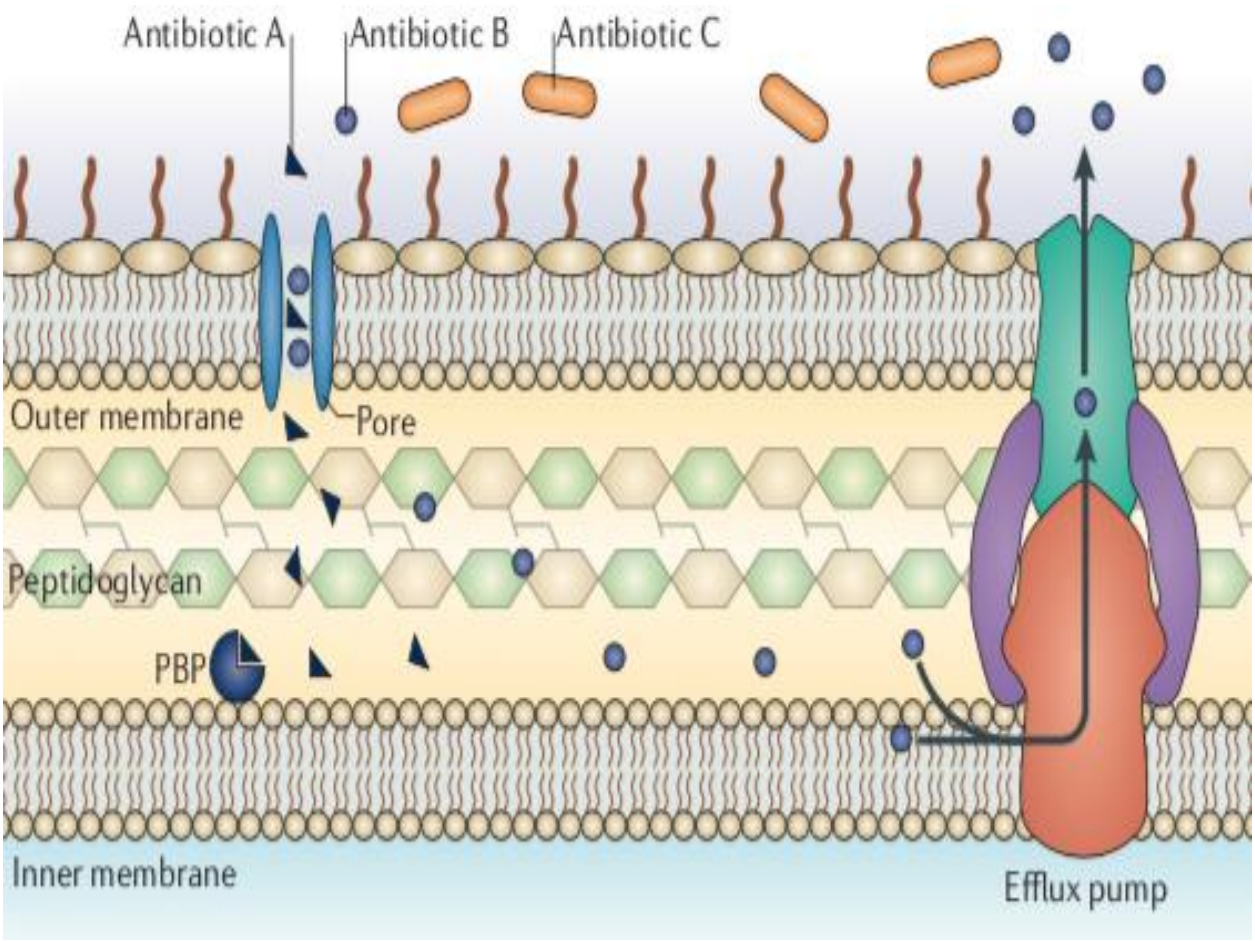

Figure 4: Intrinsic mechanism resistance depicting entry of antibiotics.

Source: Blair *et al*. (2015).

CHAPTER THREE

MATERIALS AND METHODS

The study sample covered across data obtained (n = 230) for *C. difficile* related diarrhea from the period of 1st September 2015 to 31st December 2018 from the record unit of Near East University, North Cyprus. Stool specimens were collected from patients (both in-patients and out-patients) in a non-sterile container from different departments with the consent of suspected patients. Samples were then stored at 4^0C prior to analysis. **Mini VIDAS** (Biomérieux; Serial No.: IVD 1206079) for detecting toxin A and B was used according to the procedure provided by the manufacturer. Briefly, this enzyme linked immunoassay that detect glutamate dehydrogenase (GDH); an antigen found on the surface of the toxins, is detected using optical density (OD) of 450/630 nm. Data variables used were gender, age, department and patient admittance category (in-patient and out-patient).

Statistical analysis.

The accumulated variable was analysed using Statistical Package for the Social Sciences (SPSS) software for windows version 20. Continuous data such as gender and age, were analysed as percentage of total sample collected. Categorical data such as department were analysed using Chi-square test.

Ethical Approval

This study was approved by the Health Sciences Institute Committee of the Near East University.

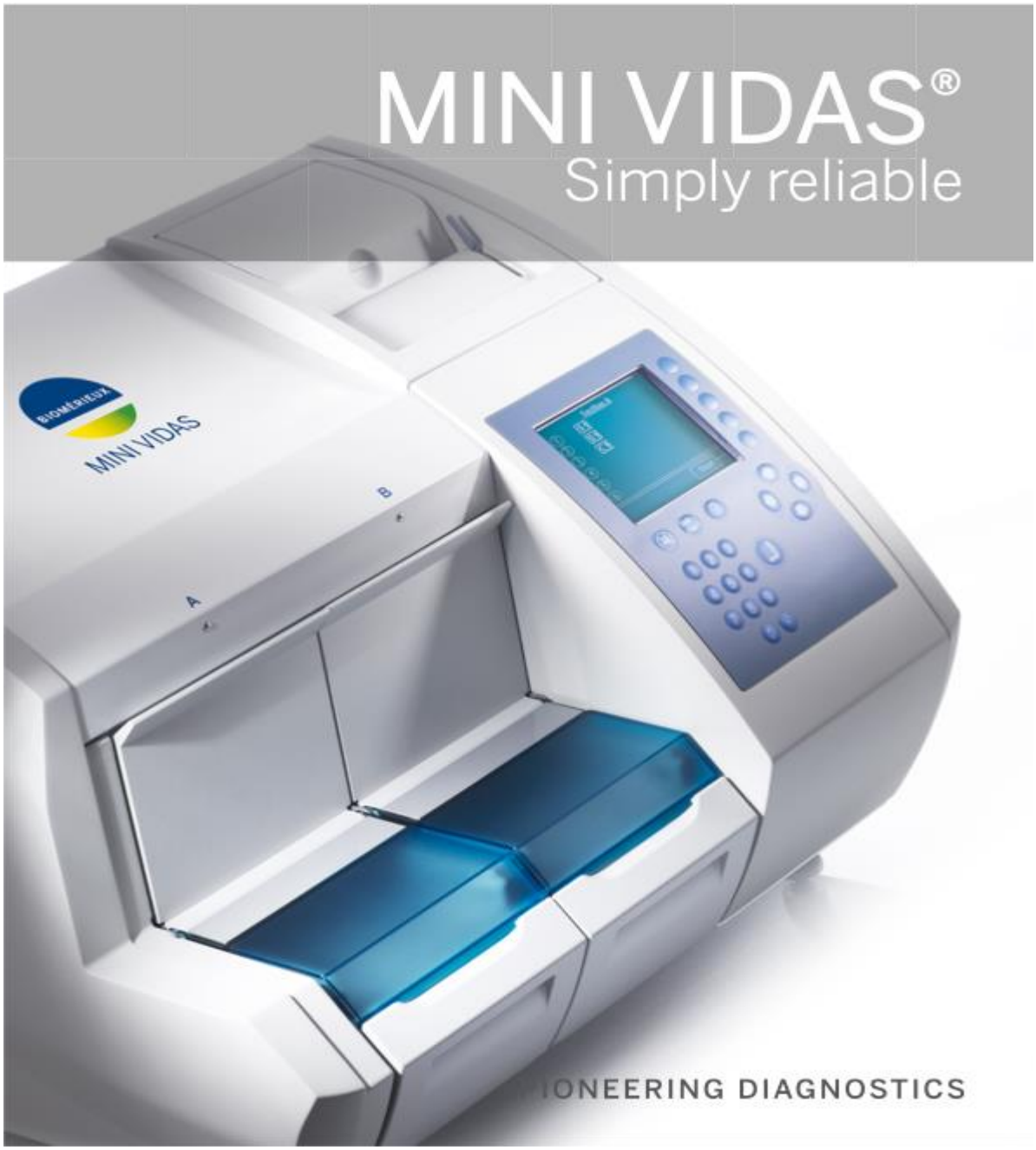

Figure 5: mini vidas biomérieux

CHAPTER FOUR

RESULTS

Table 1: Demographic and Clinical test characteristics of the patients (n = 230).

Variable	**n (%)**
Gender	
Male	108(47.00%)
Female	122(53.00%)
Age	
<20 years	15(6.50%)
20-44 years	99(43.10%)
45-64 years	58(25.20%)
≥ 65 years	58(25.20%)
Test Outcome	
Positive	37(16.10%)
Negative	193(83.90%)
Patient Admittance Category	
In-Patient	103(44.80%)
Out-Patient	127(55.20%)

Table 2: Distribution of toxin-positive and toxin-negative strains in different hospital units.

Units	**No. of *C. difficile* A-B Toxin (%)**	**No. positive A-B Toxin of *CD* (%)**	**No. of negative A-B Toxin of *CD* (%)**
Brain Surgery	2 (0.90%)	2(100.0%)	0(0.00%)
Cardiology	7 (3.00%)	2(28.60%)	5(71.40%)
Gastroenterology	30 (13.00%)	0(0.00%)	30(100.00%)
General Surgery	2 (0.90%)	0(0.00%)	2(100.00%)
Infection	26 (11.30%)	2(7.70%)	24(92.30%)
Internal Medicine	111(48.30%)	27(24.30%)	84(75.70%)
Intensive Care Unit	8 (3.50%)	1(12.50%)	7(87.50%)
Laboratory	18 (7.80%)	2(11.10%)	16(88.90%)
Pediatric	12 (5.20%)	1(8.30%)	11(91.70%)
Oncology	12 (5.20%)	0(0.00%)	12(100.00%)
Orthopedics and Traumatology	2 (0.90%)	0(0.00%)	2(100.00%)
Total	**230 (100.00%)**	**37(16.10%)**	**193(83.90%)**

Table 2 above shows the distribution of toxin-positive and toxin-negative strains in different hospital units.

The internal medicine unit recorded the highest number of *Clostridium difficile* A-B toxins of which 27(24.30%) tested positive while 84(75.70%) tested negative. This is followed by the gastroenterology unit with 30 patients out of which all tested negative with no positive

result obtained. All patients found in the gastroenterology, general surgery, oncology and orthopedics and traumatology units do not record any positive test while the only two patients found in the brain surgery unit tested all positive with no negative test result. In general, 37(16.10%) of all the 230 patients considered in the study tested positive while 193(83.90%) of them tested negative.

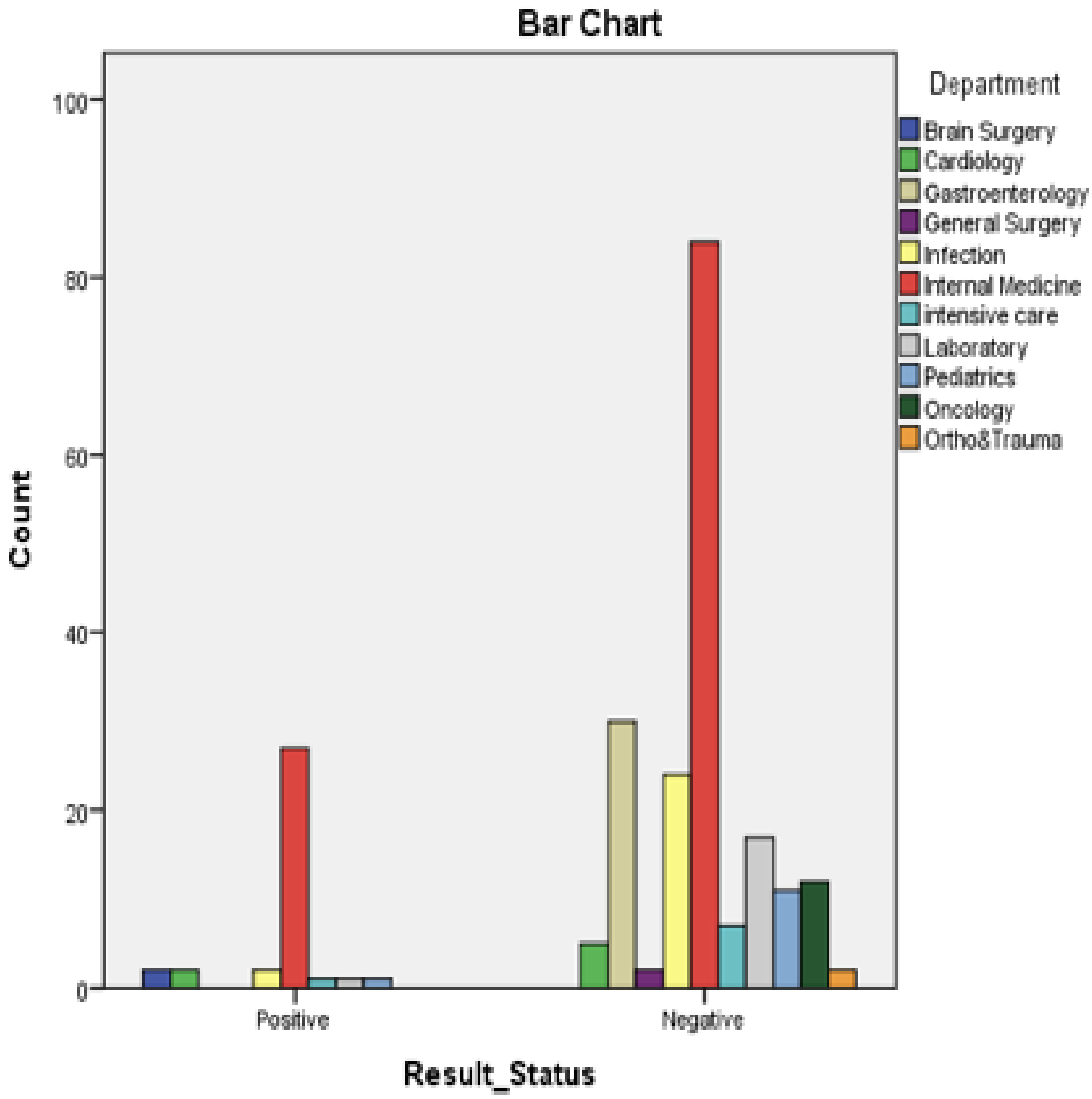

Figure 5 1: Graph Showing Distribution Pattern of Department Relative to Test Outcome

Table 3: Chi-Square Test (Gender Versus Test for *Clostridium difficile* A-B toxin)

Variable		**Positive strain n (%)**	**Negative strain n (%)**	χ^2	***p***
Gender				0.051	0.822
	Male	18 (16.70%)	90 (83.30%)		
	Female	19 (15.60%)	103 (84.40%)		

In the Gender category analysis for the result testing analysis, 18 (16.70%) of the Male patients tested positive while 90 (83.30%) of them tested negative. Just 19 (15.60%) of the female tested positive while 103 (84.40%) of them tested negative sensitive. The chi-square statistic shows that the Gender categories are not statistically significantly different in terms of test outcome (χ_2= 0.051, p<0.822). It can be inferred that gender does not have any significance association with the test result outcome.

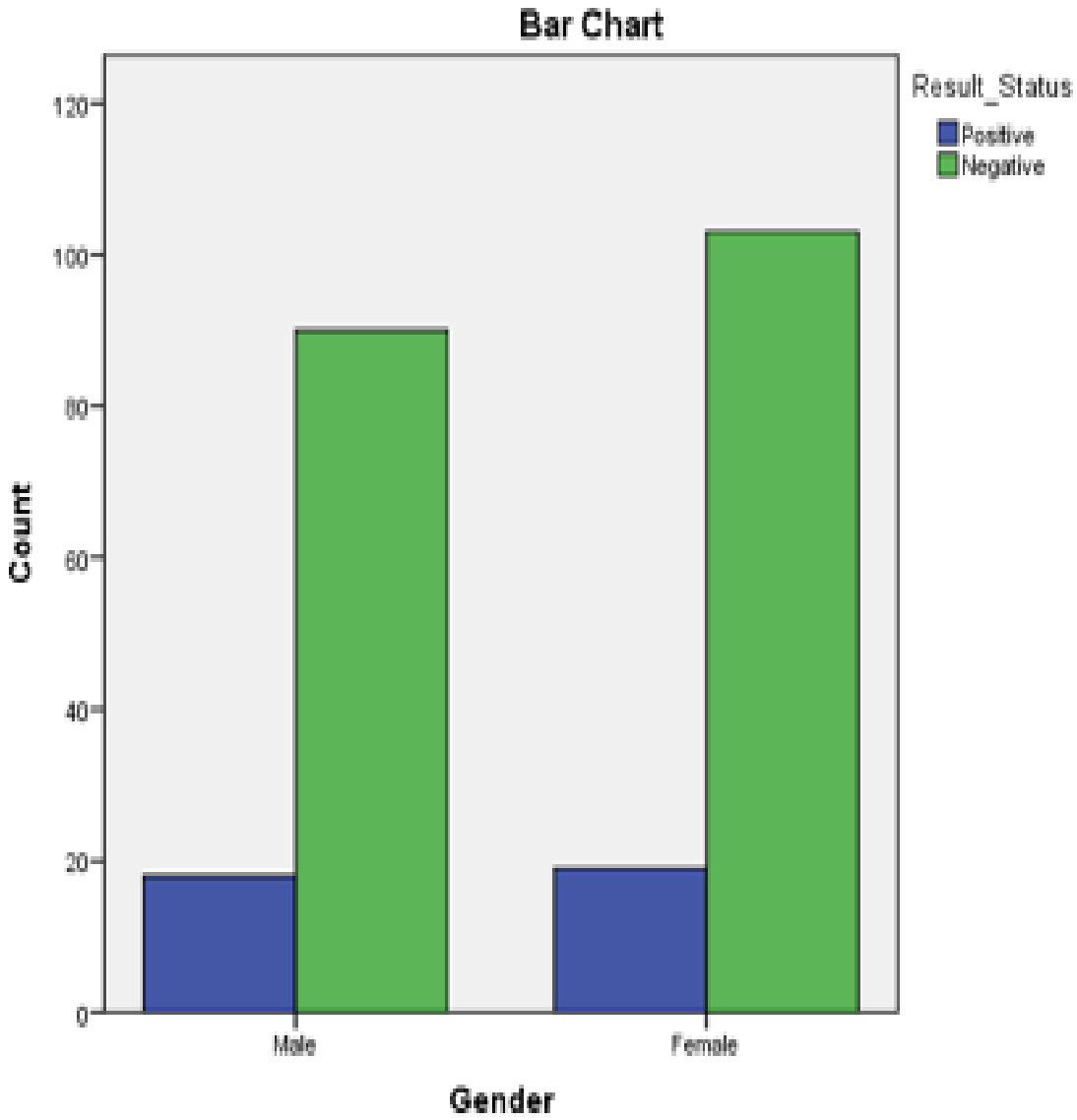

Figure 5 2: Diagram showing Gender and Test for *Clostridium difficile* Strains

Table 4A: distribution of toxin-positive and toxin-negative *c. difficile* in different age group

Age Group	**No. of *C difficile* A-B Toxin(%)**	**No. of positive *C difficile* A-B Toxin (%)**	**No. of negative *C. difficile* A-B Toxin (%)**
< 20 years	15(100.00%)	1(6.70%)	14(93.30%
20-44 years	99(100.00%)	18(18.20%)	81(81.80%)
45-64 years	58(100.00%)	9(15.50%)	49(84.50%)
≥ 65 years	58(100.00%)	9(15.50%)	49(84.50%)

Table 4B: Chi-Square Test (Age category Versus Test for *Clostridium difficile* A-B Toxin)

Variable		**Positive A-B Toxin n (%)**	**Negative A-B toxin n (%)**	**χ^2**	**p**
Age				1.336	0.721
	< 20 years	1 (6.70%)	14 (93.30%)		
	20-44 years	18 (18.20%)	81(81.80%)		
	45-64 years	9(15.50%)	49(84.50%)		
	≥65 years	9(15.50%)	49(84.50%)		

In the Age category analysis for the result testing analysis, 1 (6.70%) of the patients between 19 years & above tested positive while 14 (93.30%) of them were negative. Just 18 (18.20%) of the patients between 20-24 years tested positive while 81 (81.80%) of them tested negative. About 9 (15.50%) of the patients between 45-64 years tested positive while 49 (84.50%) of them were negative. About 9 (15.50%) of the patients from 65 years and above tested positive while 49 (84.50%) of them were negative. The Chi-square statistic shows that the Age categories are not statistically significantly different in terms of test outcome (χ_2= 1.336, p<0.721). It can be inferred that Age categories does not have any significance association with the test result outcome.

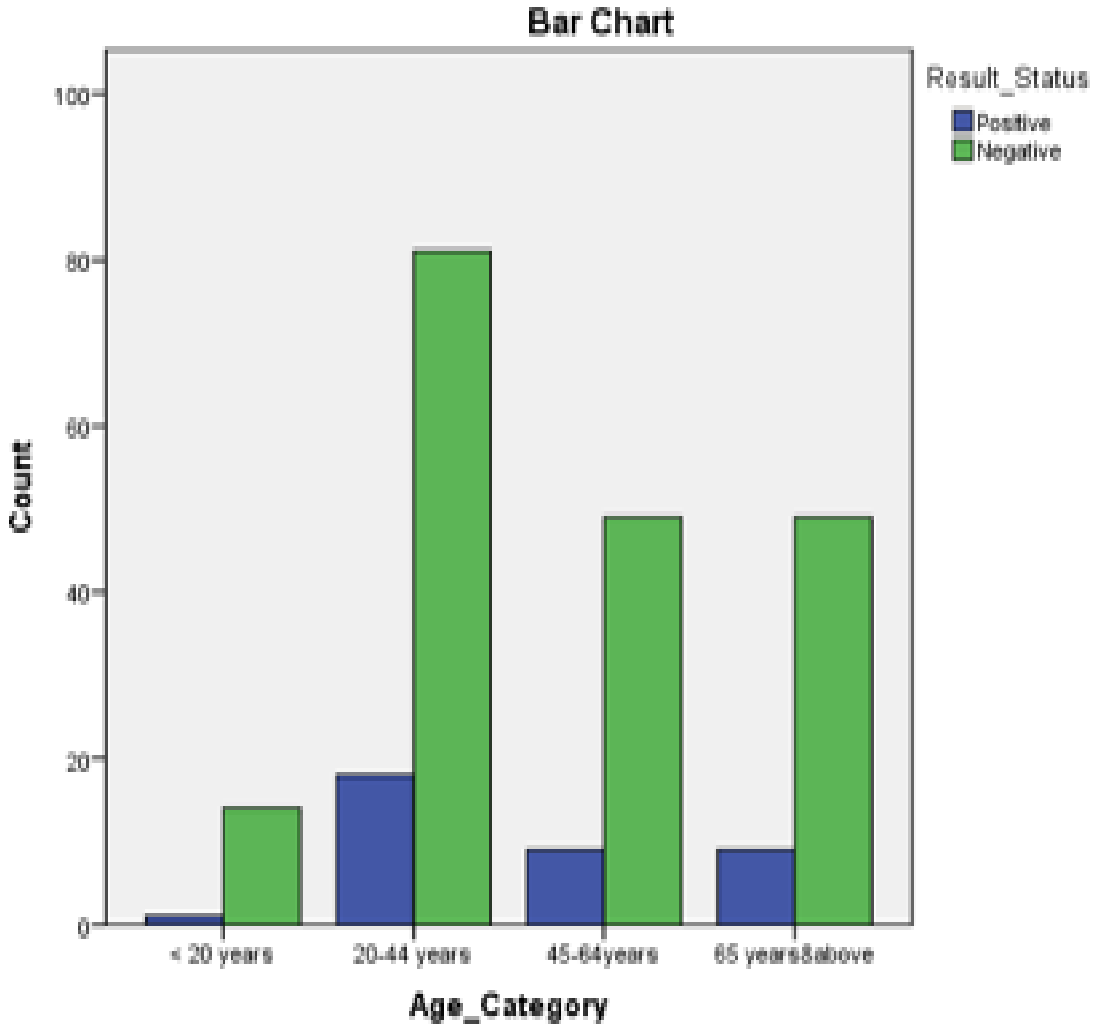

Figure 5 **3**: Diagram showing Age and test result for Clostridium difficile A-B toxin

Table 5: Chi-Square Test (Patients Admittance Status Versus Test for *Clostridium difficile* A-B toxin)

Variable		**Positive Test n (%)**	**Negative Test n (%)**	χ^2	**P**
Patient Status				5.622	0.018
	In-Patient	10 (9.70%)	93(90.30%)		
	Out-Patient	27 (21.30%)	100 (78.70%)		

In the patient admittance status category, 10 (9.70%) of the In-patients tested positive to *Clostridium difficile* A-B toxin while 27 (21.30%) of the Out-patients tested positive. Also, 93 (90.30%) of the In-patient tested negative while 100 (78.70%) of the Out-patients tested negative. The chi-square statistic shows that the patient admittance status are statistically significantly different in terms of the test outcome (χ^2= 5.622, p=0.018). It can be inferred that the condition of being admitted as an In-patient or as an Out-Patients does have significance association with the test result outcome. It can be observed from the cross-tab table that more Out-patients tested positive to *Clostridium difficile* A-B toxin than the In-patients.

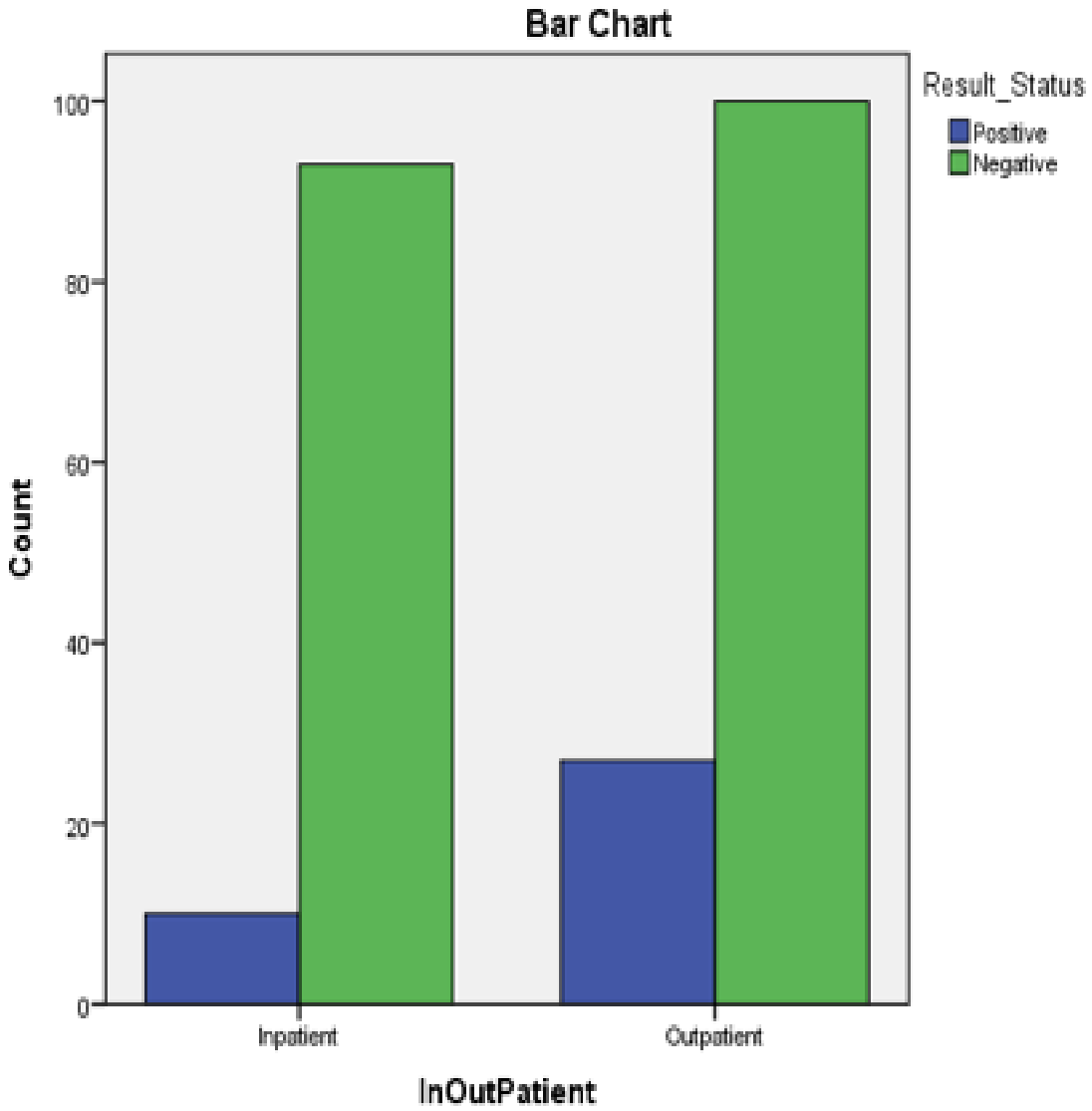

Figure 5 **4**: Diagram showing Patient Admittance Category and test result for *Clostridium difficile* A-B toxin

DISCUSSION

20-28% of recorded CDI cases in Europe and North America are community associated infections (Kuijper *et al.*, 2006). Several interventions are needed to put in place in the case of CDAD outbreak; among few is the isolation of affected patients to a particular section of the hospital or clinic, proper hygiene of wards and change/regulation of the given antibiotic. Among the major concern as a result of rise in the prevalence of this infection is the persistent rise in the use and misuse of many antibiotics. Previously, Jame et al (2018) investigated the incidence usage of antibiotics and infections that are related to health care in Northern Cyprus. The study found a statistical correlation between gender and duration of hospitalization with prevalence of health associated infections; with about 60% of inappropriate use of antibiotics. It is now necessary to investigate the prevalence of *C. difficile* toxin A-B in some parts of Northern Cyprus. This study is aimed at investigating the prevalence of *C. difficile* in Near East University Hospital.

Following the result of analysis, from table 1, it revealed that female patients' response was higher compared to male patients, while their ages category showed that 20 to 44 age groups recorded a higher percentage and less than 20 years accounted for the least percentage. 45 years and above seems to maintain constant prevalence of the infection. This study shows prevalence at lower age when compare to previous studies where high rate start at age greater than 65 (Zhou et al., 2019). Other previous studies also reported increase in severe C. difficile rate in children with bloody diarrhea (Karaaslan et al., 2016; Schwartz et al., 2014), while a study a recent study by Liao et al. (2018) reported a high prevalence of 86.36% in hospitalized adults. Another recent study by Curcio et al (2019) reported 15% prevalence

from different regions which include developing Asia, Africa-Middle East, China and Latin America. The study is a systematic literature search from various search engines and database; and comprises both community and hospital related cases.

However, in this study, patients with negative results were higher compared to those tested positive. Patients out of admission (out-patients) were higher to those patients that are on admission (in-patients). This may be due to strong and well standardized antibiotic policy adopted by the Near East University Hospital. On the other hand, Xiao et al. (2020) suggest that increase in publicity awareness among both patients and clinicians should be given necessary attention so as to curb the spread of the infection. This suggestion has since been put in place in Near East University Hospital prior to the published study.

Different units of the hospital show varying percentages of the infection with internal medicine unit recording the highest number of *Clostridium difficile* A-B toxins, but also show 75.70% of the recorded patients to be negative. Surprisingly, gastroenterology unit in our study recorded no positive result (100% negative). This is contrary to the study of Zhou et al (2019) were gastroenterology department reported a prevalence of 70.4% among patients.

From table 3 a higher percentage was seen from the male patients compared to female patients. Subsequently from both the in-patient and out-patient result for *Clostridium difficile* A-B toxin showed that those tested negatives were higher than those tested positive. And the result from cross tabulation showed no statistically significant difference between genders versus test for *C. difficile* A-B toxin, and between age categories versus test for

clostridium difficile A-B Toxin, but contrary patient admittance status was statistically significant.

Our study reported a prevalence of 16.10% C. difficile in our university hospital. This is higher than other reported studies (Prattingerová et al., 2019).The differences in incidence rate of *C. difficile* maybe due to technological advances and diagnostic expertise in different regions (Planche et al., 2013; Polage et al., 2015) and also exposure to many levels of the known risk factors.

CONCLUSION

The high increase in community-related *C. difficile* from the record unit of Near East University Hospital could be linked to the poor regulation of prescription on the use of antibiotics in TRNC. At 1 April 2016 a law was released by government: antibiotics cannot be sale without prescription. But all pharmacy still sale antibiotic without prescription (Süer et al., 2019). Other reasons may be transmission of the infection in the environment via contact and the diet consumed. Therefore, there is a need to reverse the guidelines in the use of antibiotics in TRNC. There is also a need to take representative data from all or different hospitals within North Cyprus so as to obtain a larger population, as the result of this study is limited to that of the record unit of Near East University, TRNC.

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