An-Najah National University

Faculty of Graduate Studies

MOLECULAR CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* TOXIGENIC STRAINS AND ANTIBIOTIC SUSCEPTIBLY PATTERNS AMONG PALESTINIANS IN PARTS OF NORTH WEST BANK

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Dedication

To my parents for their endless love, to my beloved husband for his encouragement and support, to my sons Qais and Abd-alrahman. And to everyone who appreciate knowledge.

Acknowledgments

I’d like to acknowledge my supervisor Dr.Walid Basha, for his wisdom , supervision, and support, our teaching team in infection control master program for their academic support, teaching assistants at An-najah national university research labs for their cooperation, and finally to defense committee members for their efforts.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

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I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Date: 1.10.2023

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Abstract

Introduction: *Clostridium difficile* is a Gram-positive, spore-forming bacillus that causes gastrointestinal sickness *C. difficile* infections (CDIs). *C. difficile* infection is considered as an opportunistic infection that develops after spores are acquired by fecal-oral route, and germinate into vegetative cells, start producing virulent toxins (A, B, and binary) that are responsible in symptoms and clinical presentation of the infection. Inflammatory diarrhea is the main manifestation among infected patients. It can be moderate and self-limiting, but it can also be severe and lead to serious complications, such as toxic megacolon and pseudomembranous colitis. Irrational use of broad-spectrum antibiotics, age above 65 years, lengthy hospital stays, and immunocompromised state, are the main risk factors for infection **Objectives**: This study aimed to molecularly characterize toxigenic *C. difficile* strains, find the prevalence of *C. difficile* and associated risk factors among Palestinians in the northern part of West Bank, in addition to antibiotic susceptibility pattern against first line choices antibiotics, and finally highlight *C. difficile* in children population. **Methods**: A cross- sectional study design was selected, a total stool diarrheal samples collected from patients in governmental hospitals, molecular characterization was done by multiplex PCR and gel electrophoresis, followed by isolation of *C. difficile* on *C. difficile* agar base with supplement, antibiotic susceptibility was performed using disk diffusion method on *Brucella* blood agar supplemented with hemin and vitamin k. **Results**: The prevalence of *C. difficile* in prevalence group was 15.7, and incidence of 5.8 per 10,000 diarrhea patient, and prevalence in children group was 10.6. Risk factors in prevalence was antibiotic and PPI use, hospitalization history and comorbidities, in the children group risk factors were antibiotic use and hospitalization history. Isolated toxigenic *C. difficile* strains was susceptible in 96 %, 89 % against vancomycin and

metronidazole respectively. **Conclusion:** 15.7 prevalence is considered as warning sign, especially when dealing with spore forming bacteria, and CDI in children group, in most cases, masked with amoeba as they result in similar presentation and treated with same antibiotic. Increased awareness about infection prevention practices in hospital and community, in addition to antibiotic stewardship programs are main recommendations to control spread of CDI

Keywords: *Clostridium difficile;* CDI; Toxins A, B; Binary; diarrhea.

Chapter One

Introduction and Literature Review

1.1. Problem statement

Clostridium difficile is a Gram-positive, spore-forming bacillus that causes gastrointestinal sickness in patients whose immune system is impaired and normal flora balance has been interrupted mainly by antibiotic use. *C. difficile* infection is considered as an opportunistic infection that develops after spores are acquired by fecal-oral route, and germinate into vegetative cells, start producing virulent toxins (A, B, and binary) that are responsible in symptoms and clinical presentation of the infection. Inflammatory diarrhea is the main manifestation among infected patients. It can be moderate and self-limiting, but it can also be severe and lead to serious complications, such as toxic megacolon and pseudomembranous colitis. Irrational use of broad-spectrum antibiotics, age above 65 year, lengthy hospital stays, and immunocompromised state, are the main risk factors for infection(1).

According to Center of Disease Control (CDC) is a significant health threat. In 2017, there were an estimated 223,900 cases in hospitalized patients and 12,800 deaths in the United States. In our region, the numbers are underestimated because of limited studies, poor testing and diagnosis of infection especially in governmental hospitals. The numbers are expected to be high as a result of the increased use of antibiotics in our Palestinian community and poor testing. A recent study revealed that about 60 % of antibiotics dispensed to without physician prescriptions (2). In addition to another study that concluded that 50 % of patients in primary care clinics reported using antibiotics for self-medication (3).

To our knowledge, there are no researches that studied *C. difficile* infection based on molecular diagnosis of toxigenic strains and described the susceptibility patterns in Palestine. Several studies reported the resistance of antibiotics among some isolate strains; the increased antimicrobial resistance in *C. diff* can increase the risk of disease development and spread. The limited number of antimicrobials for the treatment is a matter of some concern(4,5) .

1.2 Background

Clostridium difficile infection (CDI) is considered as one of the most prevalent" hospital-acquired (nosocomial) infection", and is a leading source of morbidity and mortality in hospitalized older adults. Additionally, CDI is being detected more frequently in the community and in younger individuals (6). *C. difficile* is the etiological agent of antibiotic-associated colitis, that colonizes the human intestinal tract once the usual gut flora has been disturbed (often in conjunction with antibiotic therapy).

Clostridium difficile (*C. difficile*) causes various of *C. difficile* infections (CDIs), including simple diarrhea, pseudomembranous colitis, and toxic megacolon, which can result in sepsis and even death in some cases(7). Since the beginning of the 21th century, there has been a significant increase in the incidence and severity of CDI. Managing CDI continues to be difficult due to new risk factors and disease recurrence.(8) *Clostridium difficile* can be isolated from all mammals and widely distributed in nature can be present in soli, environment, feces of humane and animals.(9)

1.3 Burden

According to CDC 2018 annual report a total of 15,591 cases of *C. difficile* infection (CDI) were reported(10). In Europe, based on the annual epidemiological report for 2016,7711 CDI cases were reported, 5 756 of which (74.6%) were healthcare-associated (HAI) CDI (11). From previous data about the epidemiology of *C. difficile* infection it is obvious that it is among the most common healthcare-associated-infection and is highly prevalent in Europe and North America. In the middle east, the surveillance of *C. difficile* is limited , in a meta-analysis study for *C. difficile* in Asia, found that the pooled incidence rates in the middle east was 11.1 %(12). A Jordanian study conducted in 2001 found that the prevalence rate of (*C. difficile*) infection among hospitalized patients was 9.7 %(13). In Palestine, a retrospective descriptive study described the characteristics risk factors and prevalence of CDI in hospitalized patients , they found that 17.7% of participants are *C. difficile* antigen positive , 13.3 % of patients were toxin A positive and 13% were positive for toxin B, in addition to increased prevalence of community-acquired CDI, which was nearly equivalent to that of hospital-acquired CDI(14).

1.4 Pathogenesis

The pathogenicity of *C. difficile* infection is dependent on toxins produced by the bacterium. Only toxin producer strains are responsible for CDI, these toxins are enterotoxin A (Toxin A) encoded by TcdA gene, cytotoxin B (Toxin B) encoded by TcdB gene . Enterotoxin causes neutrophil infiltration, inflammation, and epithelial cell necrosis by damaging actin in target cells. Cytotoxin B has been found to disrupt epithelial cells' tight junctions, increasing vascular permeability and causing bleeding(15). The discovery of naturally occurring toxin-free isolates that are nevertheless able to spread disease supports the idea that toxin A is not necessary for the virulence of *C. difficile* and toxin B does not need toxin A to function in vivo. These toxin-variant isolates have a wide range of prevalence rates over the world, typically between 0.2 and 3%. It's interesting to note that toxin A+B+ isolates induce a wide range of diseases, from asymptomatic carriage to pseudomembranous colitis. Patients infected with toxin A+B+ strains have a tendency to have more severe illness (16).

With increased severity of *C. difficile* infection, "binary toxin" (CDT) is frequently seen in *Clostridium difficile* strains (CDI) which is encoded by 2 genes *cdtA* and *cdtB*,. It induces pathogenicity through actin depolymerization, which results in the formation of microtubule-based membrane protrusions on epithelial cells, which strengthen bacterial adhesion. Many clinical investigations link binary toxin genes in *C. difficile* to higher CDI mortality. Investigations in the potential relevance of binary toxin in the pathogenesis of CDI has been sparked by the finding that the human epidemic strain types of *C. difficile*, identified by PCR ribotyping as type 027, generate binary toxin in addition to toxins A and B, also have additional modifications, including as high levels of fluoroquinolone resistance (17).

The presence or absence of these toxins forms the baseline for stool analysis when diagnosing CDI in suspected patients. *C.difficile* maybe present as part of the normal intestinal microbiota in 1–3% of healthy adults (18). Children and infants are far more likely than adults to have asymptomatic *C. difficile* infection in the GI tract.15% to 63% of newborns, 3% to 33% of babies and toddlers under the age of two, and up to 8.3% of kids over the age of two are thought to be asymptomatic carriers.

C. difficile infection presentation in children is mostly associated with mild to moderate symptoms and this possibly due to underdeveloped surface receptors for these bacteria and due to maternal antibodies acquired trans placentally or through breast milk (19,20). in addition to low prevalence of hyper -virulent strains, compared to adult(21). However, numerous recent studies showed that CDI in children is rising in both hospital and community settings, supporting the idea that the epidemiology of this disease is shifting in children (22).

C. difficile characterization is based on polymerase chain reaction ribotyping by PCR. There are several described ribotypes have been associated with CDI. The ribotypes 001, 002, 014, 046, 078, 126, and 140 have been found to be prevalent in the Middle East (23,24). Ribotype 027 was described to play a major role in its increased production of toxins, especially the binary toxin that is associated with increased infection severity (25).

1.5 Diagnosis

To establish a diagnosis of *C. difficile* infection three criteria should be present diarrhea, defined as three or more unformed stools in 24 hours, positive stool test for toxigenic *C. difficile*, and colonoscopic/ histopathologic abnormalities confirming pseudomembranous colitis (26).

Different methodologies are used to diagnose toxigenic *C. difficile* of varying specificities and sensitivities depending on manufacturers. One of these methodologies is the detection of a product of *C. difficile*, glutamate dehydrogenase (GDH), usually performed via Enzyme Immuno Assay (EIA). GDH is present in all *C. difficile* strains, so an additional toxin test is performed for confirming toxigenic *C. difficile* strain, which is also commercially available as EIA rapid test for GDH and *C. difficile* toxin/s. This method has variable sensitivity that lies between (50-90)% and specificity that ranges from 70 to 95% (1).

Binary toxin is not tested by immune chromate graphic EIA rapid test, its presence or absence can be determined by molecular techniques. Another methodology used is the nucleic Acid Amplification Technique (NAAT), including RT –PCR, loop-mediated isothermal amplification (LAMP) that targets the toxin encoding genes tcdA/*TcdB* genes. In a meta-analysis study that compare different diagnostic

methodologies, NAAT based methodologies (toxin – PCR) assays yielded the highest sensitivity (86%-92 %) and specificity (94%–97%) (27).

When testing asymptomatic carriers or cured patients, toxins do not correlate with actual *C. difficile* infection since toxin EIAs do not have the sensitivity to completely rule out *C. difficile* infection. NATs have a high sensitivity to detect toxigenic *C. difficile*, they can also identify patients who are colonized by the pathogen but do not produce any toxins (28).

1.6 Gut microbiome

About 100 trillion bacteria reside in the human gastrointestinal tract, and their genetic diversity is at least 100 times greater than that of the human genome as a whole. The microbiota is made up of these microorganisms, which also include bacteria, viruses, fungus, and protozoa. These metabolically active gut microorganisms carry out a variety of tasks, including the digestion of soluble fibers, the production of vitamins, the maturation of the immune system, and, most significantly, the prevention of pathological colonization (29). Additionally, a diverse microbiome has been linked to host health, and these microbes constantly interact with the human host. Changes in the gut microbiota's function and decreasing variety (dysbiosis) can change how it interacts with the host and immune system. These changes can also result in changes to its makeup The presence of multidrug-resistant organisms, inflammatory bowel disease, irritable bowel syndrome, asthma, allergies, metabolic syndrome, and cardiovascular disease are all linked to this disturbance. Both initial and recurrent *Clostridium difficile* infections (CDI) are influenced by gut disturbance (30).

These microbial species develop and make up the gut microbiota, creating a barrier against *C. difficile* colonization. Any changes in the gut microbial system can alter its growth and diversity. In healthy people, the microbiota typically doesn't change; nevertheless, certain factors can cause the composition to change. a variety of elements, such as the types of meals consumed, the usage of medications, the physical environment, such as travel, and even intrinsic elements, such as the immune system. The use of antibiotics is the cause most frequently cited for the disturbance of the gut microbiota resulting in diminished colonization resistance. Within days of using antibiotics, a reduction in the variety of the gut microbiota is seen, with the

compositional alterations depending on the particular antibiotic class used and the individual's microbial makeup (31). Proton-pump inhibitors (PPIs), which raise the pH of the stomach, have also been observed to have an effect on the distal gut microbiota. PPIs have been shown in vitro tests to inhibit the growth of Lactobacillus, a commensal of the mouth and gut. Additionally, several studies showed that the composition of the gut's microbes changed with PPI usage, with a decrease in *Bacteroidetes* and an increase in *Firmicutes* species which may have enhanced CDI susceptibility (32).

1.7 Risk factors

All described risk factors for CDI, are related to alteration in balance of gut microbial system as discussed previously , there are several factors that may predispose someone for getting the CDI. According to CDC the leading risk factor associated with CDI is the use of antibiotics, which makes the person 7 to 10 times more susceptible for the infection, during and one month after antibiotic course. High risk antimicrobials base on a metanalysis study for most commonly associated antibiotics with CDI and there were in the following: clindamycin, fluroquinolones ,cephalosporins, carbapenems monobactams, penicillin combinations, carbapenems, tetracyclines (33).

The antibiotic course affect the gut microbiota and disrupts the balance there causing (dysbiosis) (34). In addition to antibiotic use, there are other factors associated with increased CDI risk, these include, use of proton pump inhibitors that affect the gastric acidity that affect bacterial growth, immunocompromised patients due to impaired immune response and increased susceptibility to infection and having more severe presentations, in addition to prolonged hospitalization as CDI considered as one of most prevalent health acquired infections, due to exposure to *C. difficile* spores through contact with contaminated surfaces (35).

1.8 Treatment & resistance

For treatment of *C. difficile* infection, there are a limited choices of antibiotics that can be used in management of CDI, Vancomycin, fidaxomicin, and metronidazole are most commonly used choices. In contrast to 2014 guidelines, which suggested oral metronidazole for mild to moderate illness, 2017 IDSA and the "Society for Healthcare Epidemiology (SHEA) " updated guidelines to indicate metronidazole only for patients experiencing their first episode of non-severe CDI in situations when vancomycin or

fidaxomicin were not available (6). Fecal microbiota transplantation (FMT) which is defined as introduction of processed stool bacteria obtained from a healthy donor into the digestive tract of a patient suffering from *C. difficile* infection (36). The greatest evidence is now available for the use of FMT, in the context of recurrent CDI, which is characterized by complete symptom relief from CDI while receiving appropriate medication, followed by a two- to eight-week recurrence of symptoms 10 to 25% of individuals receiving antimicrobial therapy have recurrent CDI (6). FMT has challenges with immunosuppressed patients and requires donor screening, which is challenging in urgent situations.

1.8.1 Resistance

The main risk factor for CDI is regarded as antibiotic usage. However, because *C. difficile* is a spore-forming bacterium, spores may withstand antimicrobial therapy, germinate after treatment ends, and lead to a return of CDI. Numerous antibiotics, including aminoglycosides, lincomycin, tetracyclines, erythromycin, clindamycin, penicillin's, cephalosporins, and fluoroquinolones, which are frequently used to treat bacterial infections in clinical settings, are known to be ineffective against *C. difficile* (37).

The rapid spread of antimicrobial resistance increases the risk of CDI development and consequently it's spreading. In addition to limited antimicrobial choices, this is a global concern for CDI management, the resistance rates different and varies from one country to another, this led to a recurrence of CDI.

C. difficile resistance to routinely prescribed medicines (vancomycin and metronidazole) is a significant factor in driving epidemiological changes and the emergence of novel strain types (38). The first line of antibiotics for the treatment of CDI continues to be metronidazole and vancomycin. Most instances of CDI can still be treated with these drugs, although some strains of *C. difficile*, particularly those resistant to metronidazole, have been discovered (39,40).

Resistance to metronidazole and vancomycin as (first-line antibiotics) is rarely documented, although some reports show decreased sensitivity to metronidazole (41). These reports supported by the treatment failure following treatment , as the number of CDI cases that failed to respond to treatment has significantly increased during the

previous ten years (42).There have been several studies that reported of *C. difficile* resistance to metronidazole in various parts of the world. A study conducted in Iran, depending on the CLSI breakpoint, 5.3% of the isolates from 390 CDI patients in Iran were found to be resistant to metronidazole (40). Another study in Israel reported approximately 20.25 % resistance of *C. difficile* against metronidazole (43).

Resistance to vancomycin has been also documented but to lesser extent than metronidazole, according to another study in Iran, 8.0% of *C. difficile* clinical isolates were vancomycin-resistant(40). Based on the "EUCAST breakpoint", the percentage of vancomycin-resistant *C. difficile* clinical isolates in Israel, including 57 ribotype 027 isolates, was 47%(39). Moreover, according to a recent longitudinal surveillance research from Europe, based on the" EUCAST breakpoint "with MICs of 4 mg/liter, 2.29% of *C. difficile* bacteria were intermediately resistant to vancomycin (44).

According to the EUCAST breakpoint, a nationwide sentinel surveillance study conducted in the US discovered that 17.9% of *C. difficile* isolates were vancomycin-resistant (45).

Because luminal vancomycin is present at large concentrations in the gut (above 1,000 mg/liter in faces following oral dosing), vancomycin resistance is unlikely to have an impact on the effectiveness of the primary treatment for CDI (46).

The high-risk antimicrobials that is most commonly associated with CDI development, usually exhibited a high resistance level, with the second-generation fluroquinolones and clindamycin showing the highest levels of resistance (4).

1.9 Prevention

Given the variety of risk factors for CDI, the sources of acquisition, and the organism's strong capacity for survival, multimodal strategies are necessary to prevent CDI.

1.9.1 Hand hygiene

Hand cleanliness is essential for reducing CDI transmission due of its propensity for resistance to disinfectants and long-term environmental persistence. Nearly 60% of the time and 30% to 50% of the time, *C. difficile* may be recovered from healthcare professionals' hands and environmental surfaces, respectively (47). Hand sanitizers with

alcohol as a primary ingredient are ineffective against *C. difficile* spores. Studies have shown time and time again that soap and water washing lowers the number of spores on hands more effectively than alcohol-based hand sanitizers. It's important to note that soap and water only make *C. difficile* spore removal from hands easier. The benefits of hand hygiene have been more difficult to establish at the facility level, presumably reflecting the challenge of achieving sustained compliance on a broad scale, even though hand washing with soap and water for 15 to 30 seconds definitely reduces spore load (48).

Recent recommendations emphasize the use of soap and water specifically in environments with a high incidence of CDI, while allowing the use of either soap and water or alcohol-based cleansers for normal hand hygiene in environments with a low incidence of CDI. Nevertheless, given the ineffectiveness of alcohol-based sanitizers, the majority of hospitals still advise hand washing with soap and water for any patients with CDI, regardless of local incidence rates (49).

1.9.2 Contact precautions

According on regional CDI incidence estimates, the "Infectious Diseases Society of America"(IDSA) recommends a different length of contact precautions. After symptoms have subsided, isolation is advised for at least 48 hours; however, in high-incidence settings, isolation may last longer and even result in discharge. Based on data that *C. difficile* shedding may continue for at least 1 to 4 weeks following therapy, the latter recommendation for prolonged isolation was made. (50). Additionally, Patients with *C. difficile* infection often contaminate various body sites (chest, abdomen, hands, and arms), ambient surfaces (bed rails, tables, telephones, and call buttons), and hospital equipment. The use of contact precautions with gown and gloves is intended to reduce the transfer of *C. difficile* to healthcare provider's hands, clothing, and equipment through contact with these surfaces. Despite microbiologic plausibility, evidence for therapeutic efficacy at reducing CDI rates is less certain (51).

1.9.3 Antibiotic steward ship program

Even while almost all antibiotics raise the risk of CDI, but the drugs that consistently carry the highest risk of CDI include clindamycin ,fluroquinolones, cephalosporins, and carbapenems (52). Additionally, short-term perioperative antibiotic use carries the same

risk (53) . The risk of CDI is decreased by making an effort to limit antibiotic exposure or by choosing the agent with the lowest risk. One of the most efficient ways to lower CDI rates is through antimicrobial stewardship. The establishment of an "antimicrobial stewardship program" can lower the incidence of CDI by as much as 24 to 60% (54). An antibiotic stewardship, which targeted restriction use of fluroquinolones, clindamycin, amoxicillin/clavulanate, and cephalosporins, was found to be successful in reducing the development of multidrug-resistant pandemic ribotypes of *C. difficile*, such as 001 and 027 (55).

In addition to antimicrobial stewardship interventions that aimed at perioperative antibiotic prophylaxis have been shown to significantly lower CDI rates. perioperative prophylaxis-focused stewardship treatments can lower CDI rates by up to 71% (56). An efficient antimicrobial stewardship program aims to maximize the desired antibiotic impact while minimizing unfavorable effects (such as the risk of CDI or other multidrug-resistant bacteria). These factors include antibiotic selection, timing of administration, and duration. Key interventions include avoiding prolonged antimicrobial prophylaxis or longer than recommended treatment courses, choosing antibiotics that are effective for the intended use without being overly broad-spectrum, and avoiding unnecessary antibiotic administration (such as for the treatment of asymptomatic bacteriuria or viral respiratory infections) (57). And concerning surgical prophylaxis the IDSA offers recommendations on the choice, administration, and duration of perioperative prophylactic antibiotics (58). As these medications carry the highest overall risk, avoiding carbapenems, fluroquinolones, or clindamycin whenever possible may assist to lower the risk of CDI. Almost never should prophylaxis last longer than 24 hours, and the most recent Centers for Disease Control (CDC) recommendations clearly state that extra antimicrobial prophylaxis doses should be avoided after the surgical site has been closed (58).

1.10 Objectives

This study aimed to:

- Estimate prevalence of toxigenic *C. difficile* infection among Palestinians in parts of North west Bank.
- Describe *C. difficile* infection based on molecular method among Palestinians in parts of North west Bank.
- Compare between immunochromatographic EIA rapid tests and PCR methods for diagnosing *C. difficile* infections.
- Describe antibiotic susceptibility pattern of resistance in isolated strains.
- Describe risk factors associated with the infection in our community.
- Describe *C. difficile* infection in children according to age groups.

Chapter Two

Methods

2.1 Study design, time and settings:

Cross sectional study design was used to characterize toxigenic strains of *C. difficile* and to describe pattern of isolated strains among Palestinians in parts of North West bank, the study was conducted at An-najah national university for molecular analysis and susceptibility testing, during the period between Jun 2022 to March 2023.

2.2 Study population, sample and sample size

Study population defined as Palestinians in parts North west bank (Tulkarm, Nablus, and Jenin), Study target population includes patients' samples with diarrhea, in previously determined governates. A convenience sample of all available stool samples (that meets criteria) in previously mentioned governates hospitals including governmental and tertiary care hospitals were collected. Sample size calculated according to formula for cross sectional studies/ surveys for qualitative variable, as follows: north west bank in previously determined governates.

$n = (Z1 - \alpha/2)^2 . P \div d^2$, with

n= sample size,

Z1- α/2 =Statistical constant that corresponds confidence level , at 5% type 1 error (p value < 0.05), this constant equal 1.96

P: expected prevalence in the population based on previous studies, this value was determined to be 10 %, based on metanalysis study for *C. difficile* in middle east, and a study of *C. difficile* prevalence in Jordan (12,13).

d: absolute error, selected to be at 5 %, after substitution of these variables in the equation above, sample size equals 138.

2.2.1 Inclusion criteria

- Diarrheal samples with /without blood for 3 or more in 24 hours.

2.2.2 Exclusion criteria

- Any stool sample that doesn't meet the definition above, like formed, contaminated samples.

- Patients with recent laxative use.

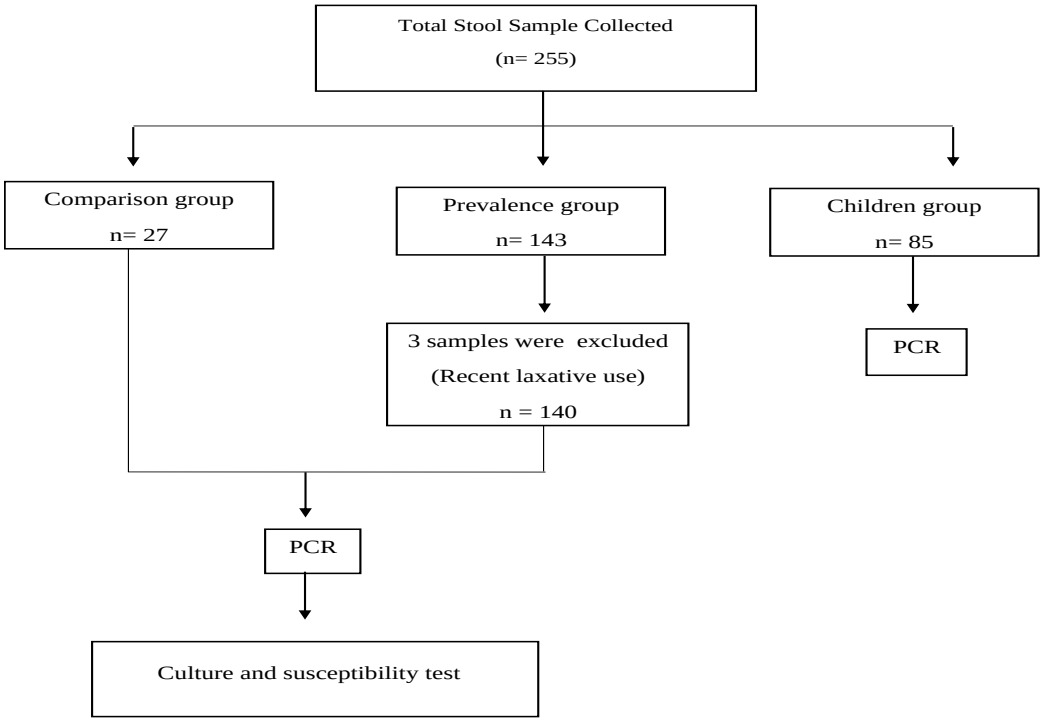
2.2.3 Sample collection and storage

After getting approval for sample collection from hospitals, the previous criteria were given to technicians in hospital labs, in order to collect samples for our study. Collected stool samples were stored at 4 °C, until molecular and susceptibly testing performed. Refrigeration was the best storage condition for *C. difficile*. Refrigeration was selected as storage condition for study samples, after referring to a study that compare different storage conditions (room temperature, refrigeration (4 °C) and -70 °C for all testing techniques for *C. difficile* diagnosis including culture, PCR, and EIA. It was concluded that refrigeration of stool samples for *C. difficile* diagnosis is advised for both short and long term(up to 60 days) storage (59).

The study sample is divided into three sub-samples, first one ,was positive samples that were tested by rapid immunochromatographic EIA, it was used as comparison group, for comparison purposes with PCR in order to validate the procedure to be used in the second part which was, unknown samples to be tested by PCR for prevalence calculation and risk factor analysis. The third part of sample was from children, it was separated from the second part in order to be analyzed separately from part 2, because *C. difficile* in children population is different from adult population as discussed in introduction. Susceptibility testing was performed for all positive isolates from first and second group, because most of symptomatic diarrhea children was diagnosed with amoeba based on microscopic examination and they were on metronidazole therapy that affect the culture results.

Figure 1

sample work flow



This figure demonstrates study sample classification, the total stool samples collected were grouped into 3 sub- groups. The first one for comparison, the second for prevalence and risk factors and the final one was children group. All groups were tested by PCR, and only the first and second groups was cultured.

2.3 Study variables

- Main study outcome according to which samples classified as accepted or rejected : diarrhea, as defined before.

- Dependent variables:

1. *C. difficile* infection, defined by toxigenic strain based on type of toxin as determined by multiplex PCR

2. Antibiotic susceptibility result, which determined by disk diffusion method

- Independent variables: Ant biotic use, PPI use , hospitalization, comorbidities.

- Back ground and clinical variables:

1. Age in the study was classified into age groups as following:

- Children sub-sample:

1. 2 - < 5 yrs.
2. 5 - < 10 yrs.
3. 10 - < 14 yrs.

- Age group in prevalence group:

1. Adolescents or teenagers (14-<18years).
2. Adults (18 - ≤ 65 yrs.)
3. Older adults (> 65 yrs.)

- Antibiotic use**:** based on CDC definition of antibiotic use was included, if the patient was on, or finished an antibiotic course, from the high-risk list, during a month before onset of symptoms.

- Immunocompromised: was considered in patients with, cancer, chemotherapy, immunosuppressive drugs.

- Previous hospitalization: defined as hospitalization in prior 180 day before the current visit or admission (60).

- In addition to, other variables like gender, comorbidities as diabetes, cardiovascular and gastro intestinal pathologies as ulcerative colitis or inflammatory bowel disease, and proton pump inhibitor use (PPI).

1. Severity classification: according to IDSA/SHEA 2021 classification:

- Non- sever *C. difficile*, mild to moderate cases in which WBC count of less than, 15000 cells/mL and a serum creatinine level less than 1.5 mg/dL , diarrhea is the only presentation
- Sever *C. difficile*, one of the following findings, WBC count greater than 15,000 cells/mL or serum creatinine more than 1.5 mg/dL at presentation.
- Fulminant *C. difficile*, when rapid deterioration signs like hypotension, shock, ileus, or toxic megacolon are present.
- Carrier state definitions in our study, carriers are classified into
- Carrier of non-toxigenic strain, GDH positive , toxin negative, and the expression " carrier" was used
- Asymptomatic colonizer of toxigenic strain: GDH positive, *C. difficile* toxin/s positive, and had no symptoms, and expressed as asymptomatic colonizer.
- All the previously mentioned variables were taken from patient's records; confidentiality of patient's data was maintained and secured based on IRB approval.

2.4 Measurement tool and data collection

2.4.1 Stool DNA extraction

Collected stool sample for this study, were prepared for multiplex PCR , by DNA extraction, using special kit for stool DNA extraction, from (Qiagen, Hilden, Germany).

The DNA was extracted from stool samples according to manufacturer hand book included with the kit for DNA purification form stool specimens, the extraction protocol used was the "isolation of DNA from Stool for Pathogen Detection protocol". This kit contains special "InhibitEX Tablet" which was added each sample, to adsorb inhibitory enzymes that are present in stool sample matrix, which may affect PCR reaction. After completing the procedure, the extracted DNA from isolation columns, is ready for multiplex PCR reaction. The extracted DNA was stores at -20 until PCR procedure performance (61).

2.4.2 Bacterial DNA extraction

The isolated *C. difficile* colonies were subjected to DNA extraction using a different DNA extraction kit from "Promega", the protocol for DNA extraction from gram positive bacteria was used according to manufacturer's instructions (62). And isolated DNA was stored at -20, until PCR performance. Bacterial DNA extraction step was added as additional identification step for isolated colonies.

2.5 PCR

Multiplex PCR followed by agarose gel electrophoresis method was selected for molecular characterization of toxigenic *C. difficile* strains. A multiplex PCR was used to detect toxin- encoding genes: tcdA for toxin A , tcdB for toxin B , (cdtA, cdtB) for binary toxin . In addition to 16S primer always used as initial control for bacterial DNA presence . The glutamate dehydrogenase (GDH) gene is used as a second check, validating the identification of *C. difficile*. The gluD PCR detects the gluD gene, which codes for glutamate dehydrogenase (GDH), which is only found in *C. difficile*. GDH is a metabolic enzyme that all *C. difficile* strains produce in large amounts (63).

Table 1

Primers Sequence for C. difficile multiplex PCR

Name	Target	Sequence	Amplification
tcdA-F	TcdA gene	5'-GCATGATAAGGCAACTTCAGTGGTA-3'	629
tcdA-R		5'-AGTTCCTCCTGCTCCATCAAATG-3'	
tcdB-F	TcdB gene	5'-CCAAARTGGAGTGTTACAAACAGGTG-3'	410
tcdB-RA		5'-GCATTTCTCCATTCTCAGCAAAGTA-3'	
tcdB-RB		5'-GCATTTCTCCGTTTTCAGCAAAGTA-3'	
cdtA-FA	cdtA gene	5'-GGGAAGCACTATATTAAAGCAGAAGC-3'	221
cdtA-FB		5'-GGGAAACATTATATTAAAGCAGAAGC-3'	
cdtA-R		5'-CTGGGTTAGGATTATTTACTGGACCA-3'	
ctdB-F	cdtB gene	5'-TTGACCCAAAGTTGATGTCTGATTG-3'	262
cdtB-R		5'-CGGATCTCTTGCTTCAGTCTTTATAG-3'	
PS-F	16S-rDNA	5'-GGAGGCAGCAGTGGGGAATA-3'	1062
PS-R		5'-TGACGGGCGGTGTGTACAAG-3'	
GluD-F	gluD gene	5'-GTCTTGGATGGTTGATGAGTAC-3'	158
GluD-R		5'-TTCCTAATTTAGCAGCAGCTTC-3'	

.

2.5.1 PCR reaction

Table 2

PCR reaction mixture

Hot star master mix	12.5 µl
tcdA-F	1 µl
tcdA-R	1 µl
tcdB-F	0.4 µl
tcdB-RA	0.2 µl
tcdB-RB	0.2 µl
cdtA-FA	0.05 µl
cdtA-FB	0.05 µ
cdtA-R	0.01 µl
cdtB-F	0.01 µl
cdtB-R	0.01 µl
PS-F	0.05 µl
PS-R	0.05 µl
GluD-F	0.1 µl
GluD-R	0.1 µl
H2O	6.7 µl
Total	22.5 µl

PCR mixture for one sample , to which 2.5 µl of extracted DNA was added, to get final volume 25 µl for each sample. Primer concentration: 50 pmol/µl.

2.5.2 Multiplex PCR program (Thermal cycles)

The PCR mixture that prepared according to the previous table, was entered the PCR machine, based on specific thermal cycles program based on the used protocol. see appendix A- table A 1

2.5.3 Interpretation

The presence or absence of the various toxin genes in the strains can be detected based on the distinctive banding pattern on gel. Positive samples of produce bands for corresponding genes as presented in the table A 2 in appendix A.

According to this table which demonstrates amplification size for each gene that guided interpretation of DNA bands on gel electrophoresis. (See appendix B, figure B1)

With each gel electrophoresis run a ladder or marker for DNA fragments size , was placed in the first well of gel , according to this DNA ladder the amplification size of the bands was reported.

The GDH determines whether *Clostridium difficile* is present or not, as it is considered as a characteristic marker for *C. difficile*, while the 16S determines whether or not DNA is present.

For each a stool sample that contains *Clostridium difficile* bacteria there are two bands that needed to be present, one for the 16S - r DNA as a marker for presence of bacterial DNA, and the second band for GDH enzyme coding gene, which is a characteristic enzyme for the *Clostridium difficile* bacteria.

The stool samples that have toxigenic *Clostridium difficile* strain, additional bands corresponding to the toxin type needed to be present on the gel.

2.6 Stool culture and Susceptibility testing

2.6.1 Stool culture

Collected and stored stool samples regardless the sub- class that the sample belonged to, were prepared for culture. A spore germination step using alcohol shock method for 1 hr. was performed, prior culturing on *C.difficile* ager. This step supported and enhanced the sensitivity of culture and isolation of *C.difficile* from stool samples. Then the samples were cultured under anaerobic conditions, using anaerobic gas generating kit, in palladium catalyst jar, to achieve strict anaerobic conditions, for successful *C.difficile* isolation, and incubated at 37 °C, for 48 hour (63 ,64). Isolated bacterial colonies, were identified, first by gram and spore stain, and secondly by multiplex PCR and gel electrophoresis, preceded by bacterial DNA extraction, based on the previously mentioned procedures.

2.6.2 Culture media

In our study for the initial isolation of *Clostridium difficile* from fecal materials, *Clostridium difficile* Agar Base was utilized. The medium's ingredients are chosen to promote *C. diff* luxuriant growth, and to be selective at the same time , as the majority of Enterobacteriaceae, *Enterococcus faecalis*, *Staphylococci*, Gram-negative non-sporing anaerobic bacilli, and *Clostridia* (except *Clostridium difficile*) that may be found in significant numbers in fecal samples are all inhibited by the selective agents D-cycloserine (500 g/mL) and cefoxitin (16 g/mL). Isolated *C. difficile* colonies had Grey-white, elevated, uneven, and opaque appearance. The supplement was added to media

after autoclaving together with 7 % V/V horse / sheep blood at 50 °C. The composition of *C. difficile* agar bas with supplement is the same composition of "Cycloserine Cefoxitin Fructose Agar "CCFA" which is considered as the selective media for *C. difficile* isolation.

2.6.3 Susceptibility testing

Before susceptibility testing, refreshing subculture was performed for the isolates, on 5 percent blood agar plates in an anaerobic environment, as mentioned previously for 24 hours. *Brucella* blood agar supplemented with hemin and vitamin K media was used for susceptibility testing, using disk diffusion method. The inhibition zones for the selected antibiotics, was taken from two studies that compare between disk diffusion and MIC, and established an inhibition zone range for each antibiotic, with correlation with CLSI and EUCAST epidemiological cut-off values (ECOFFs), and it was 23 mm for metronidazole and, 19 mm foe vancomycin ,and there were no major errors when comparing using disk diffusion method and E- test methods and it was below 2% (65, 66). Vancomycin, and metronidazole were selected for susceptibility testing. Vancomycin and metronidazole were selected as they are the mainstay antibiotics used in management of *C. difficile* infection, in addition to some reports of emergence of reduced susceptibility. Cultured isolates were suspended in thioglycolate to a density of 1.0 McFarland. A sterile cotton swab will be placed in the suspension to transfer inoculum, and spread equally all over surface of the plate. Plates were incubated in anaerobic atmosphere, antibiotics concentrations that were used vancomycin (5 µg), metronidazole (5 µg) (40,66).

2.7 Quality control

With each run of samples positive and negative controls were performed, in order to provide a procedural check and validation of results. Positive stool sample for all genes, used as positive control. In addition to use of suitable molecular sized DNA ladder, for proper gel banding interpretation.

2.8 Safety

Safety issues were taken into consideration, when dealing with clinical stool specimens, isolated colonies. In addition to adherence to general lab safety precautions when dealing with flammable reagents and biohazard material.

2.9 Statistical data analysis plan

Frequencies were used to describe distribution of *C. difficile* and other variables in sample. Statistical analysis for patient's data taken from records and patient's results was performed using SPSS version 21. Significance level was established at 0.05. The relation between the infection and risk factors based on data taken from patient's records, as antibiotic use, immune status, age group etc..., were tested by Chi –x2 test. In addition to multinomial regression model for statistically significant variables on univariate analysis (Chi –x2), to determine the actual association between dependent and independent variables.

2.10 Ethical approval

This research proposal was submitted to the university IRB committee, and get their approval, in addition to contact with Palestinian ministry of health, contact with some special hospital separately to get their acceptance for sample and Patient's data collection. Confidentiality of patient's information was maintained and secured. Samples given codes; no names were used.

Chapter Three

Results

3.1 Results for sub-sample 1 (comparison group)

A total of 26 positive samples and 1 was GDH positive toxin negative by immunochromatographic rapid test, were collected from tertiary care hospitals, the sample were re- tested by multiplex PCR method as described in methodology chapter, and the results were as following:

Table 3

PCR/ immunochromatographic rapid test comparison

Result	Immunochromatographic rapid test	Multiplex PCR
Positive	26	22
Negative	0	5
Carrier	1	0
Total	27	27

The percentage of matched result between two methods was determined by using following formula (positive by PCR / positive by rapid test) * 100 %. = 22/26 * 100 % = 84.6 %, and percentage of discrepancy in results = (100 –84.6) = 15.4%. One sample was GDH positive by rapid test, and it was negative by PCR. In addition to binary toxin that detected by PCR and not detected by EIA rapid test, it was recovered from 2 samples of 26 (7.7%) of positive samples by PCR.

3.2 Result for sub- sample 2 (prevalence group)

3.2.1 Demographic data

Table 4

Age, gender and C. difficile infection

Characteristics		Frequency, n (%)	Positive *C. difficile*, n (%)	Negative *C. difficile*, n (%)	Carrier, n (%)	P- value
Age group n=140	Adolescents (14-<18) yrs.	23 (16.4)	3 (13)	20 (14.3)	0	0.283
	Adults(18- ≤65) yrs.	84 (60)	10 (11.9)	72 (51.4)	2 (2.4)	
	Old adults (>65 yrs.)	33 (23.6)	9 (27.3)	24 (17.1)	0	
Total		140	22	116	2	
Gender n=140	Male	72 (51.4)	10 (13.9)	61 (84.7)	1 (1.4)	0.828
	Female	68 (48.6)	12 (17.6)	55 (80.9)	1 (1.5)	
Total		140	22	116	2	

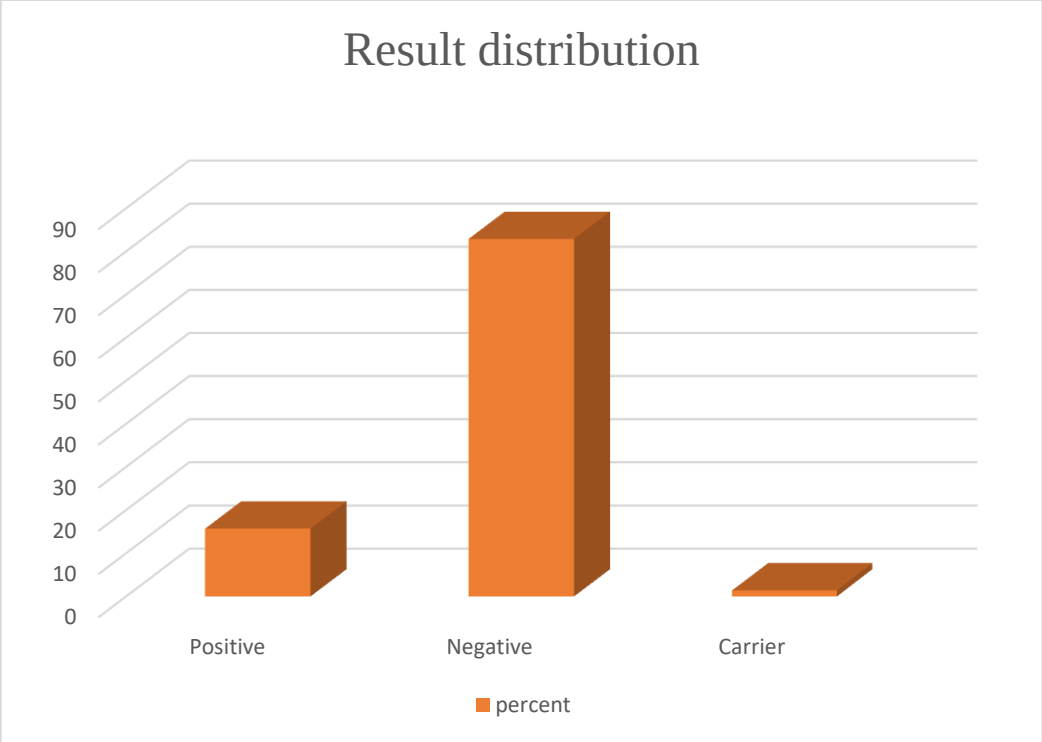
This table presents age group and gender distribution in the study sub- sample 2, in addition to their association with *C. difficile* infection, 60% of samples age lies in the second age group, which is young adult ranging from 18 to 64 years, and about 23.4%, 16.6 % for old adults and adolescents respectively. In addition to approximately similar gender distribution between males and females was found. At established significance, p value < 0.05, there were no significant association between age , gender and risk of infection(P- values: 0.283, 0.828) respectively.

The residency distribution in sub- sample 2 was: 37.9 % (n= 53) of samples referred to patients from Tulkarm, 33.5 %, (n=47) from Nablus, and the remaining 28.5 %, (n=40) were from Jenin (See appendix B, figure B 2)

3.2.2 *C. difficile* infection results by PCR for sub- sample 2

Figure 2

result distribution for sub-sample 2

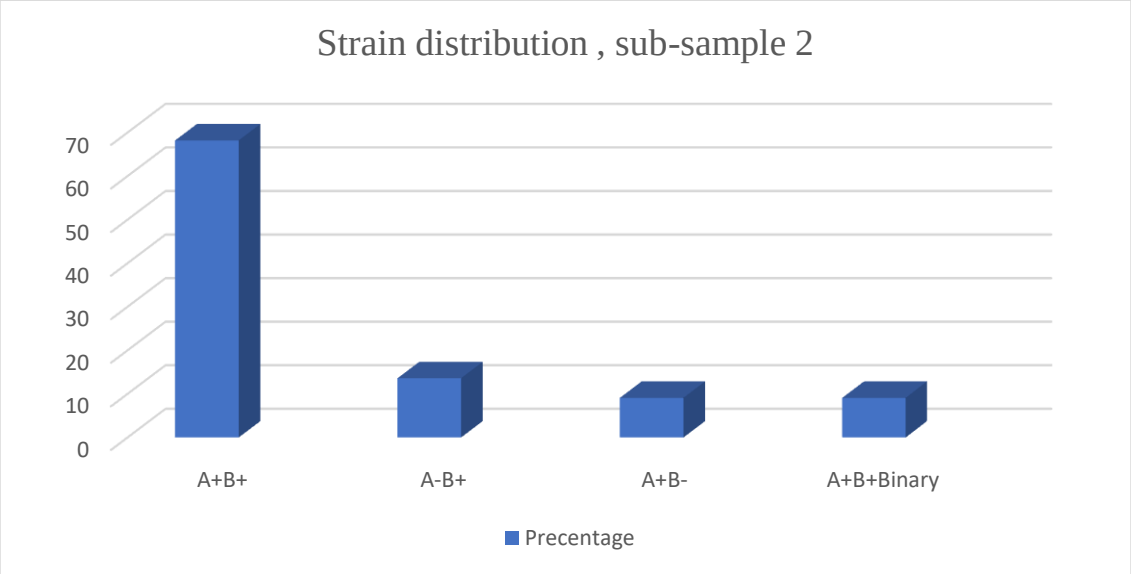


This figure demonstrates result distribution in sub-sample 2 for multiplex PCR, percentage of toxigenic *C. difficile* strain was 15.7 %(n=22), 82.9% of samples were negative for *C. difficile* infection(n= 116), and 1.4 % (n=2) of samples were carrier *C. difficile*. Carrier was defined as sample that was positive for GDH, and negative for all toxins.

3.2.3 Molecular characterization of positive toxigenic strains

Figure 3

Stain distribution, sub -sample 2

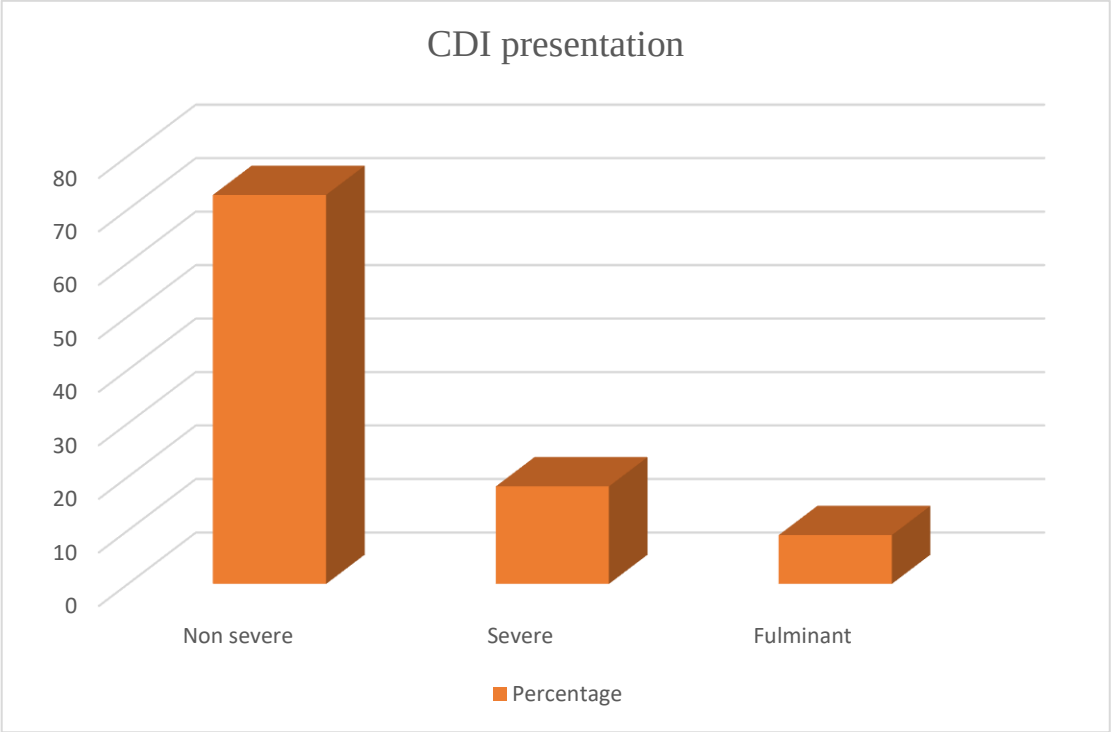


The previous chart (figure 3) demonstrates molecular typing of toxigenic *C. difficile* strains and their distribution in percentages from total positive results. From 22 total positive , A+B+ strain was the most prevalent strain , accounting for 68.2 % of strains (n=15). The variant strain A-B+ was 13.6 % (n=3), and A+B- variant was 9.1%, (n=2). The binary toxin was recovered from 2 samples accounting for 9.1 % of toxigenic strains.

3.2.4 Infection presentation

Figure 4

CDI presentation sub – sample 2



In figure 4 the infection presentation was classified into non- severe which, include mild to moderate cases, severe and fulminant *C. difficile*, based on definitions in methodology chapter. 72.7 Percentage of patients had non sever CDI (n=16), 18.2 % were severe(n= 4) and fulminant CDI was present in 9.1 % of patients, (n=2).

3.2.5 Antibiotic use and *C.difficile* infection

Table 5

Antibiotic use and C. difficile infection

Result	Use antibiotic (%)	Didn't use antibiotic, n (%)	Un known, n (%)	Total n (%)	P – value
Positive, % from use	18 (27.7%)	4 (5.6%)	0	22 (15.7%)	
Negative	46 (70.8 %)	66 (93%)	4	116 (82.9%)	0.0010
Carrier	1 (1.5%)	1 (1.4 %)	0	2 (1.4%)	
Total	65 (100 %)	71 (100%)	4	140 (100%)	

Table 7 above shows that 50.7 % (n=71) didn't use antibiotics, and 46.5 % (n=65) of patients used antibiotic based on antibiotic use definition in methodology chapter, in addition to 4 patients with un known antibiotic use history due to incomplete records, and these 4 patients had negative result. *C. difficile* was positive among approximately 28 % of patient who use antibiotics, and absent among 93 % of patients who didn't use antibiotics.

P- value 0.01, at established significance level, there is a significant association between antibiotic use and *C. difficile* infection. The antibiotic classes consumed at least 1-month prior onset of diarrhea symptoms among *C.difficile* positive patients (18 of 22) 82 %, the distribution of used antibiotics in decreasing order was, Cephalosporins, carbapenems, Fluroquinolones, aminoglycosides, Piperacillin-tazobactam and, clindamycin in 33.3 % (n=6) , 22.2 % (n=4) , 16.7 % (n=3), 11.1% (n=2), 11.1% (n=2), and 5.6 %(n=1) respectively. (See appendix B, figure B 3).

3.2.6 Hospitalization history and *C. difficile* infection

Table 6

Hospitalization history and C.difficile infection

Result	Yes ,n (%)	No , n (%)	Unknown history, n (%)	Total n (%)	P – value
Positive, n (%from Hospitalization history)	16 (31.4 %)	6 (6.9 %)	0	22 (15.7 %)	
Negative, n (% from Hospitalization history)	33 (66%)	79 (92.9%)	4	116 (82.9 %)	.001
Carrier, n (% from Hospitalization history)	2 (3.9 %)	0	0	2 (1.4 %)	
Total n (%)	51 (100%)	85 (100%)	4 (2.1%)	140 (100)	

From this table, as defined in methodology chapter, hospitalization history was reported among 30 % of patients with positive *C. difficile*. Additionally, 93 % of patients with no previous hospitalization history, was *C. difficile* negative. And 3.9 % of patients who was carrier for *C. difficile* had hospitalization history.

P -value = 0.001, at established significance level, there is a significant association between previous hospitalization history and *C. difficile* infection

3.2.7 Comorbidities and *C. difficile* infection

Table7

Comorbidities and C. difficile infection

Result	No comorbidities, n (%)	Chronic, n (%)	IBD, n (%)	Immunosuppressed, n (%)	Total, n (% from result)	P – value
Positive, n (%from comorbidities)	3 (5.4)	7 (11.7)	2 (100%)	10 (45.5)	22 (15.7)	
Negative ,n (%from comorbidities	52 (92.8)	52 (86.6)	0	12 (54.5)	116 (82.9)	
Carrier ,n (%from comorbidities	1 (1.8)	1 (1.7)	0	0	2 (1.4)	0.001
Total, n (%from comorbidities	56 (100)	60 (100)	2 (100)	22 (100)	140 (100)	

From this table, 40 % (n=56), and 60 % (n=84) had comorbidities, and they were distributed in 42.9%, 15.7 %, 1.4% for chronic, immunocompromised and Inflammatory Bowel Disease (IBD). *C.difficile* was present among 45.5 %, 11.7 % patients who had immunocompromising compromising , chronic comorbidities. Tow patients had IBD, and both were *C. difficile* positive (100%) . 3(5.4%) patients who had no comorbidities were *C. difficile* positive .

P -value = 0.001, at established significance level, there is a significant association between comorbidities and *C. difficile* infection

3.2.8 PPI use and *C. difficile* infection

Table 8

PPI use and C. difficile infection

Result	Use PPI ,n (%)	Didn't use PPI, n (%)	Total n, (% from result)	P – value
Positive, n (% from PPI use)	20 (25.6)	2 (3.2%)	22 (15.7)	
Negative, n (% from PPI use)	57 (73.1)	59 (95.2%)	116 (82.9)	0.001
Carrier, n (% from PPI use)	1 (1.3)	1 (1.6%)	2 (1.4%)	
Total, n (% from PPI use)	78 (55.7)	62 (44.3)	140 (100)	

Distribution of PPI use among patients in sample was 55.7 % (n=78), *C. difficile* infection was positive among 25.6 % of patients who use PPI , and it was negative in 95.2 % in patients who didn't use PPI .

P -value = 0.001, at established significance level, there is a significant association between PPI use and *C. difficile* infection.

3.2.9 Multinomial regression

The independent variables that yielded a significant association (p value < 0.05) in univariate analysis (Chi-x2), was entered a multinomial regression model, the variables were, antibiotic use (p value 0.001), PPI use (p value 0.001), comorbidities (p value 0.001), previous hospitalization (p value 0.001). see appendix A, table A 3.

The multinomial regression model p-values was 0.004, 0.03, 0.001, 0.0014 for antibiotic use, PPI use, hospitalization history and comorbidities, respectively. The model strength was 90.1%.

All variables in the regression model were statically significant and they were significant in both univariate and multinomial analysis, so it was concluded that all previous factors were associated with *C. difficile* infection risk.

3.2.10 Prevalence and incidence rates calculation

A total 22 positive samples of 140, with 116 negative and two samples were carrier For prevalence, the following formula was used:

Prevalence = No. of case / population at risk.

= 22/ 140 * 100 = 15.7 %

Incidence rate of *C. difficile* among diarrhea population

For determination population at risk, we refer to studies that describe prevalence of diarrhea in general population in Palestine or near countries, and then relate it proportionally to our target population (Jenin, Nablus, Tulkarm). Target population count was determined by referring to population counts for 2023 as published on Palestinian Central Bureau of Statistics website, which equals 990405. In addition, diarrhea incidence in general population was taken from a coessential study in Gaza

that characterized diarrhea among Palestinian population, in time between 2017-2018, and the incidence of diarrhea in general population was 3.8 per 100 induvial. Using these numbers, diarrhea population in our study setting was calculated, which was= 37635.39.

No. of cases determined by our study were 22 cases.

Incidence per 10,000 =(22/ 37635.39)* 10,000.

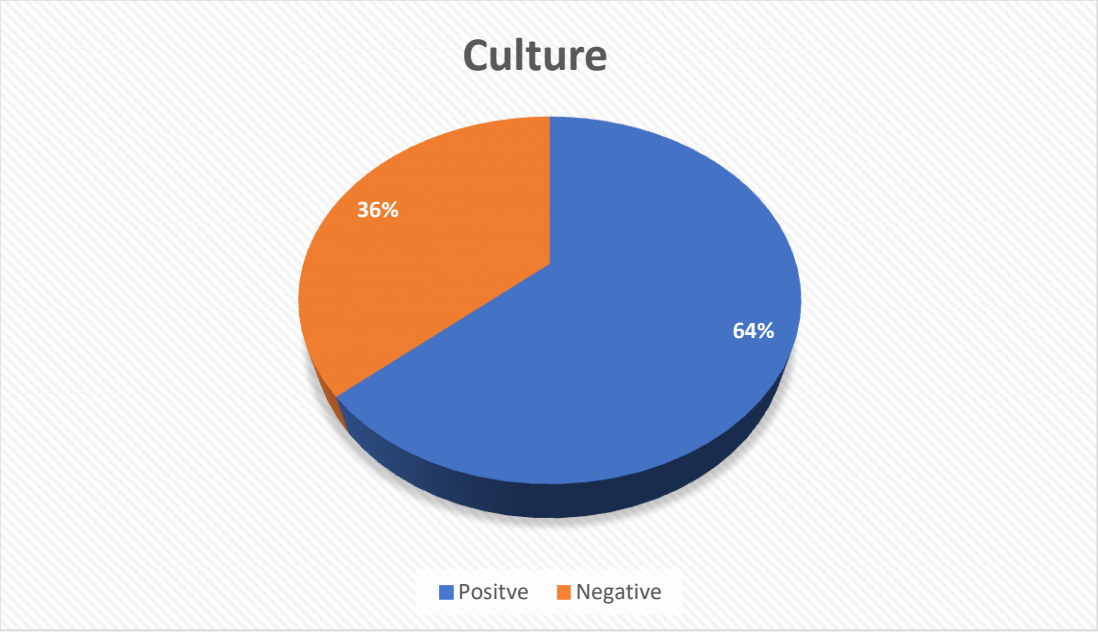
Incidence of toxigenic *C.difficile* infection among Palestinians in parts of north west bank = 5.84 per 10,000 person with diarrhea.

3.3 Culture and susceptibility results

C. difficile colonies were recovered from toxigenic strains of symptomatic patients in sub-sample 1 and 2, out of 44 positive samples confirmed by PCR, 28 samples were recovered by culture, using the culture protocol discussed in methodology chapter, further identification of colonies was preformed preliminary by gram, and spore staining and confirmed by PCR. Then susceptibility testing was performed, and the results were as following:

Figure 5

culture results for toxigenic C. difficile strains from sub-sample 1, 2



C. difficile was recovered from 28 sample, with a recovery rate about 64 %, and the remaining 36% was negative culture(n=16).

3.3.1 Antibiotic susceptibility testing results

Figure 6

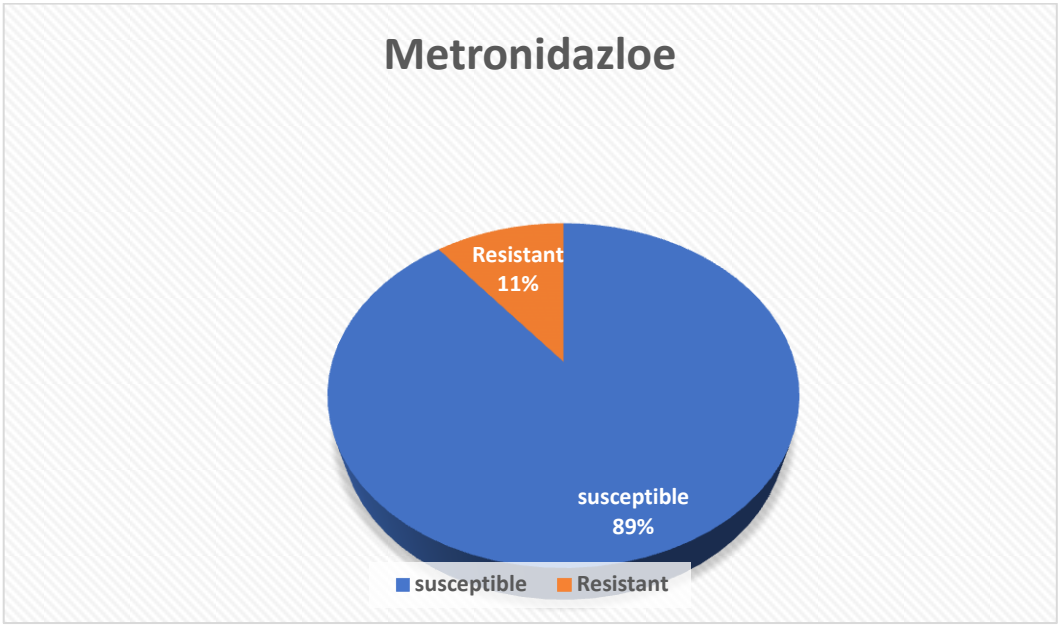
Susceptibility of C.difficile to Vancomycin

In figure 6, 96 % (n= 27) of *C. difficile* colonies was susceptible for vancomycin and 4 % (n=1) were vancomycin resistant, vancomycin disk concentration was 5 µg, Inhibition zone (19 mm) ” based on EUCAST breakpoints”. The vancomycin resistant isolate was A+B+ Binary +.

3.3.2 Susceptibility of *C.difficile* to Metronidazole

Figure 7

Metronidazole susceptibility



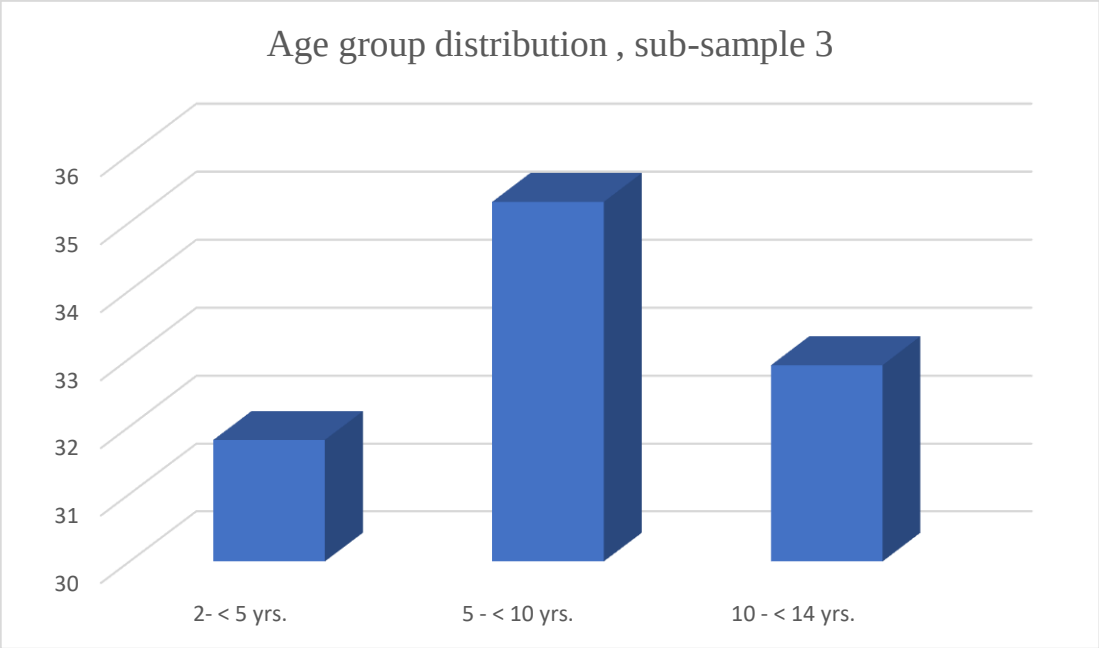
This figure illustrates *C. difficile* susceptibility to metronidazole, about89% (n=25) of isolates were susceptible to metronidazole, and 11% (n=3) were metronidazole resistant, the two resistant isolates were binary toxin positive. Metronidazole disk concentration was 5 µg, inhibition zone 23 mm based on "CLSI breakpoints". The two of three resistant isolates were belonged to A+B+ Binary +, and the remaining resistant isolate was A+B+ strain.

3.4 Results for sub- sample 3 (children group)

3.4.1 Demographic data

Figure 8

Age group distribution in sub-sample 3

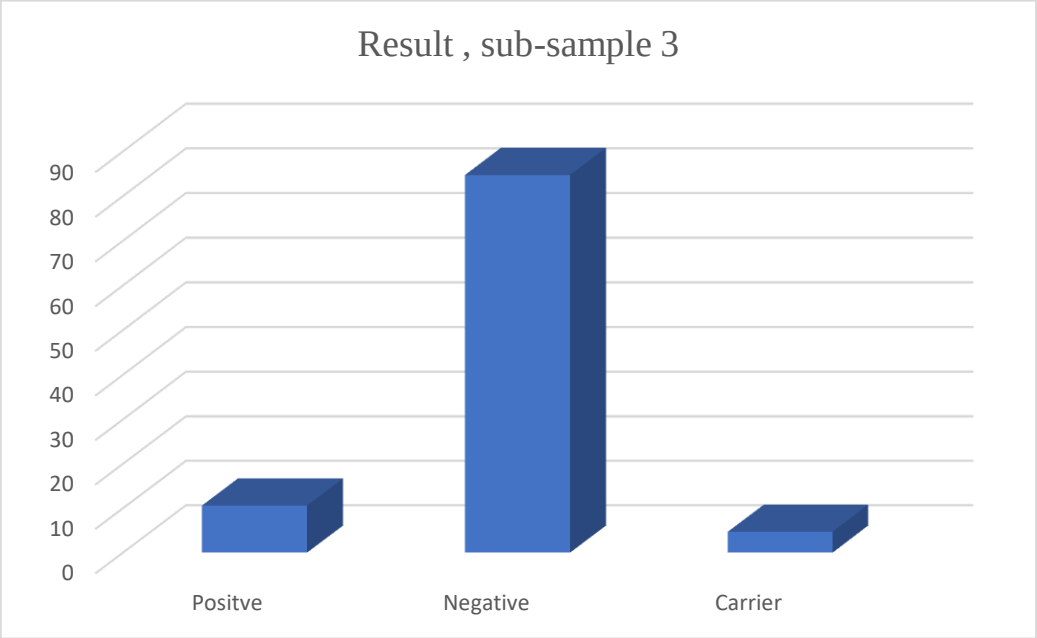


In figure 8, age group was categorized three groups, the first was 2- < 5 yrs. and it was 31.8% (n= 27), the second age group was ages between 5 - <10 yrs., and it composed 35.3% (n= 30) of the children sub sample, the remaining age group was 10 - < 14 yrs. that represented 32.9 % (n= 28). The gender distribution in children sub- sample was about 48 % (n=41) % for males, and about 52 %(n=44) for females. Residency was distributed as following 37.7 % (n=32) of samples in children group were from Nablus, 32.9 % (n=28) were from Tulkarm and remaining 29.4 % (n= 25) were from Jenin. (See appendix B, figures B 4, B 5).

3.4.2 Result distribution in sub-sample 3

Figure 9

Result distribution in sub-sample 3



In figure 9 the results were distributed as following : 10.6 % (n=9), 84.7 % (n=72), and 4.7 % (n=4), for positive ,negative and carrier , respectively. Over all prevalence of *C. difficile* in children group 10.6 %. The *C. difficile* positive toxigenic strains were distributed as following 66.7% (n=6) for, 22.2% (n=2) and 11.1 % (n=1) for A+B+ , A-B+ and A+B- respectively . See appendix B, figure B 6.

3.4.3 Result by age group for sub-sample 3

Figure 10

Result by age group, sub-sample 3

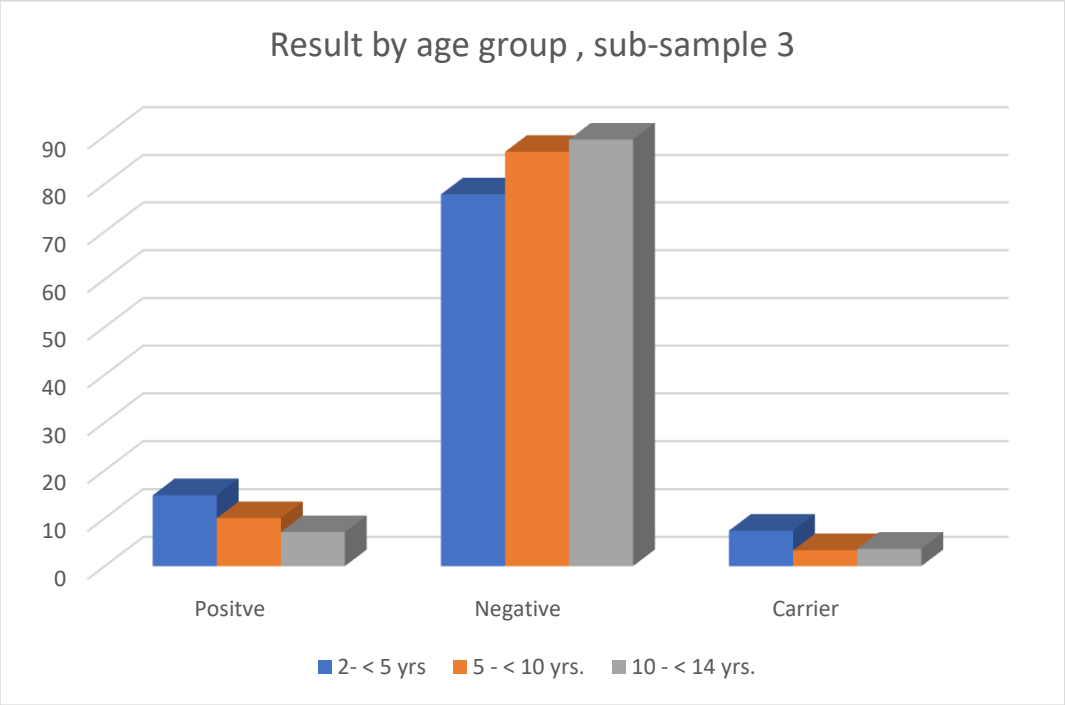


Figure 10 demonstrates the result of distribution of sub -sample 3, positive toxigenic strains was present among 14.8 % (n=4) of the first age group 2- < 5 yrs. (n =27), and it was declining in the remaining groups, 10 % (n= 3) from the second age group (5- < 10) (n= 30), and the final age group had (n= 28), 7.1 % (n=2) of positive toxigenic *C. difficile*.

The carrier status composed 7.4 % (n=2) of the first age group, 3.3 % (n=1) and 3.6 % (n=1) of the second and third age groups respectively.

3.4.4 Antibiotic use and *C. difficile* infection

Table 9

Antibiotic use and C.difficile infection, sub-sample3

Result	Use antibiotics n (%)	Didn't use antibiotics n (%)	Unknown n (%)	Total n (% from result)	P-Value
Positive, n (% from antibiotic use)	7 (23.3)	2(3.8)	0	9(10.6)	
Negative, n (% from antibiotic use)	23 (76.7)	47(88.7)	2	72(84.7)	
Carrier, n (% from antibiotic use)	0	4(7.5)	0	4(4.7)	
Total, n (% from antibiotic use)	30(35.3)	53(62.4)	2(2.4)	85 (100)	0.03

As shown in table 13 above, 62. 4 % (n=53) didn't use antibiotics, and 35.5 % (n=30) of patients used antibiotic, in addition to 2 patients with unknown antibiotic use history. *C. difficile* was positive among approximately 23 % (n=7) of patient who use antibiotics, and absent among 89 % (n=47) of patients who didn't use antibiotics.

P- value 0.03, at established significance level, there is a significant association between antibiotic use and *C. difficile* infection.

Cephalosporins (second and third generations), amoxicillin clavulanic acid, and ampicillin were most common antibiotic classes found in antibiotic use history.

3.4.5 Hospitalization history and *C. difficile* infection

Table 10

Hospitalization history and C.difficile infection, sub-sample 3

Result	Hospitalization history n (%)	No hospitalization history n (%)	Unknown n (%)	Total n(% from result)	P-Value
Positive , n (% from hospitalization history)	6(22.2)	3(5.4)	0 (0)	9(10.6)	
Negative, n (% from hospitalization history)	18 (66.7)	52 (92.9)	2(100)	72 (84.7)	
Carrier, n (% from hospitalization history)	3(11.1)	1(1.8)	0	4(4.7)	
Total, n (% from hospitalization history)	27(31.8)	56 (65.9)	2(2.4)	85 (100)	0.037

As presented in table 14, hospitalization history was reported among 22 % (n=6) of patients with positive *C. difficile*. Additionally, about 93 % (n=52) of patients with no previous hospitalization history, was *C. difficile* negative. In addition, 11 % of patients who was carrier for *C. difficile* had hospitalization history.

P -value = 0.037, at established significance level, there is a significant association between previous hospitalization history and *C. difficile* infection.

Chapter Four

Discussion & conclusion

4.1 Comparison group

This study aimed to molecularly characterize toxigenic strains of *C. difficile* using multiplex PCR method. The first step in our study was to evaluate the molecular methodology by comparison with positive samples tested by immunochromatographic rapid test that routinely used for diagnosis of *C. difficile*. The result of collected positive samples was blinded, and it was taken after testing by multiplex PCR in order to compare between two methods.

The results between two methods were compatible by 84.6 %, and discrepancy was 11.8 %. One sample (3.7%) tested by rapid test and was GDH positive and toxin negative, and when tested by PCR it was negative. The discrepancy in results between two methods is expected, due to differences in sensitivity and specificity between two methods, this leads to either false positive rapid test result, or false negative PCR results. Some studies indicates that testing *C. difficile* in patients who receive laxatives, increase false positive results, and this factor was controlled in our study by excluding patients with laxative use history, but it wasn't excluded in rapid test patients so it may contribute to false positive results in rapid test (67).

In addition to variable specificity in immunoassay test, that ranges from 70 to 95% and this results in 5- 30 % false positive results, and there are group of factors related to sample nature that may affect rapid test result like blood, mucus that commonly seen in diarrheal samples. Despite high sensitivity of molecular technique 86 -92 %, the false negative results remain possible, about 7- 14 % (1,27). The advantage of PCR method over rapid test is detection of binary toxin, and confirmation of GDH positive, toxin negative result. Additionally, PCR reaction may be affected by inhibitor enzymes in stool samples, that may interfere with the DNA polymerase enzyme, and this factor was controlled using special DNA extraction from stool samples kit, in which there was a special inhibitory capsule added to extraction mixture to get rid of inhibitory enzymes in stool samples. False positive results in both methods may arise from testing asymptomatic patients and this can be excluded by limiting testing to symptomatic patients

4.2 Prevalence of *C. difficile* infection and associated risk factors

The overall prevalence of *C. difficile* infection among Palestinian adults in our study was 15.7 %, which is similar to findings of meta-analysis study of prevalence in developing countries which was 15 % (95 %CI : 13-17) % (68). The percentage of present study is also near to finding of studies in Jordan in 2009 which was 13.7 %(69), in addition to recently published study in Iran that found 12.3 % prevalence of *C. difficile* (70), and the retrospective Palestinian study in 2018 , which found that prevalence rate 15.2 % (90/593) (14). In our study the incidence rate of CDI among diarrhea patients was 5.84 per 10,000 persons with diarrhea, which lies in the pooled range of the previously mentioned meta-analysis study, in which the incidence rate of CDI was 8.5 per 10,000 patient-days (95% CI 5.83–12.46) (68).

The intestinal mucosa can be colonized by "non-toxic *C. difficile* "NTCD " strains, which are frequently isolated from asymptomatic people (71). In our study 1.4 % of prevalence sub-sample was carrier of non- toxigenic strains, that most commonly not associated with diarrhea and the cause of presentation of these patients may be related to other causes, in a study in united kingdom found non toxigenic *C. difficile* colonization rate among healthy adults was between 4 % and 7% (72).

C. difficile infection is associated with group of risk factors, including age > 65 years, antibiotic use, PPI use, hospitalization history, and underlying comorbidities(1). According to CDC reports and several studies age > 65 years is associated with increased risk for *C. difficile* infection (73). In our study age was not statistically significant (P- value 0.283), and this is similar to findings of retrospective study in Palestine(14). A study in 2018, concluded that age had limited association with *C. difficile*, and the fact behind this lies in the indirect effect of aging and increased risk for comorbidities, increased chance of hospitalization and consequently antibiotic use, and the previously mentioned considered as factors associated with increased risk for *C. difficile* (1,74).

In addition to individual differences between different population distribution and demographic composition of communities. The population composition of Palestinian community is different from other western and European communities which according to Eurostat web site, the > 65 years age group compose 20.3 % of total population in

2019. In addition to increased contact of this age group in nursing homes, but the situation in our Palestinian community is different, and the percentage of > 60 years by mid-2022 in our Palestinian community was about 5 .5% according to "Palestinian Central Bureau of Statistics (PCBS)" (75).

Moreover, there are several studies that indicate increased incidence of *C. difficile* among children.

Gender in our study and in several previous studies, isn't associated with increased risk for CDI (P-value 0.828) (2).

The other risk factors, antibiotic use, hospitalization, comorbidities and PPI use, all were significant with same P-value, 0.001(<0.05) . These findings were consistent with published literature (1,14,35), and the theory behind this associated with the integrity of microbiome, which form barrier that protect against " colonization "with *C. difficile*.

The effect on microbiome due to antibiotic use through bactericidal activity of antibiotic courses, which consequently leads to decreased resistance to colonization, which promotes the development of pathogenic microorganisms like *C. difficile* and alters the composition of the individual's microbiota(31).

A significant factor affecting *C. difficile* proliferation is pH. Low gastric pH prevents *C. difficile* spores from sporulating, but high gastric pH and an alkaline intestinal pH promote *C. difficile* survival and permit sporulation and germination of the bacterium's vegetative phase. According to this data, acid suppression treatments like PPIs encourage the survival and proliferation of vegetative *C. difficile*, which raises the risk of contracting CDI. According to the study's findings, strains of *C. difficile* grew more readily in alkaline colonic pH than they did in acidic colonic pH. This is supported by the high prevalence of CDIs in patients with alkaline stools because low gastric pH prevents *C. difficile* spores from sporulating. (76,77)

The microbiome is regarded as a crucial component of the innate immune system and has been shown to be disturbed in a number of diseases. The composition of the gut microbiota can change, altering its function and diminishing its diversity, which can alter how it interacts with the host and immune system. This disruption is associated

with metabolic syndrome, cardiovascular disease, irritable bowel syndrome, asthma, allergies, and inflammatory bowel disease. (30)

Increased exposure to spores is the factor behind hospitalization history as a risk factor for CDI, which classified as hospital acquired infection. Additionally, *C. difficile* incidence rates are considered as marker for safety of hospital environment and quality of medical service in the health care facility(60). In our study hospitalization history was significantly associated with CDI risk, (P-value 0.01).

Although some studies indicates that any antibiotic therapy, regardless the class, has the potential to alter the usual gut flora, which could allow *C. difficile* spores to germinate and create its toxins(26) . In our study sample antibiotic classes, which were found among positive *C. difficile* patients, were as following: cephalosporins, carbapenems, fluroquinolones, aminoglycosides and clindamycin. All of which were described to be associated with CDI risk (33).

Strain distribution is different between regions, most prevalent in toxigenic strain of our study, was A+B+ stain that composed about 70 % of strains, the strain variants A-B+ was 13 %, the strains A-B+ and A+B+ Binary+ were similarly distributed about 9 % for each strain. Our strain distribution was similar to that described in Iran (78). Binary toxin prevalence is different between regions and it ranges from 1.6% to 34.6% of clinical *C. difficile* (79). In our study, percentage of binary toxin is considered as low prevalence when compared with other countries as Israel, in which the binary toxin containing hyper virulent strain that contain binary toxin are the ribotype 027 strain that contains binary toxin has spread throughout Israel and is now the most prevalent strain percentage (39).

Presentation of *C. difficile* infection ranges from mild self-limiting (non -severe), severe and fulminate disease. Our results show that about 73 % of CDI patients had non severe infection, 18 % had sever presentation and 9 % had fulminate CDI. Severity of infection presentation is governed by host factors related to immune defense and having severe presentations in patients with immunodeficiency (80), in addition to type of toxin; several studies link the hyper- virulent strains that produce binary toxin associated with more sever presentations of CDI (17).

4.3 Culture and susceptibility

C. difficile was cultured and isolated using *C. difficile* ager base with supplement, which is similar in its composition to "CCFA", under anaerobic condition , as described in methodology chapter, our recovery rate was 63 %, which is considered as acceptable when compared with published recovery rate for "CCFA" 69 % (81).

Oral vancomycin and metronidazole are considered as first line antibiotic choices for management of *C. difficile*, recent studies reported emergence of resistant *C. difficile* strains especially binary toxin containing strains , which was lower than other strains in the study sample (41) and this interpret high resistance rates in regions, which have high incidence rates of hyper virulent strains which contains binary toxin as described earlier in Israel (39) , in our study 96% of isolates were susceptible to vancomycin, while 89 % (n=25) of isolates were metronidazole susceptible and 4 % (n=1) , 11 % (n= 3) of isolates had reduced susceptibility against vancomycin and metronidazole, respectively. In meta-analysis study found pooled resistance rates of metronidazole and vancomycin, which was 3%-7% for metronidazole and 1% - 4% for vancomycin (82). Resistance rates to metronidazole was higher than the pooled range in the meta-analysis study, but is lower than several regions that recorded higher rates (83,39), and vancomycin resistance rates was on the upper limit the range, but generally our susceptibility results were acceptable, and vancomycin ,metronidazole still effective against *C. difficile*. The resistant isolates were A+B+ Binary and A+B+. One strain was resistant to vancomycin and metronidazole that was A+B +Binary , that belong to patient from the comparison group that had recurrent episode and this support that recurrence and resistance nature of binary containing strains.(84)

4.4 *C. difficile* in children population

Despite limited information on CDI in pediatric patients, *C. difficile* is becoming more well acknowledged as a significant pathogen in kids. Additionally, a number of studies have revealed an increase in CDI among kids in both community and hospital settings. (85,86)

The importance of *C. difficile* in babies is still debatable. Numerous studies have shown that in healthy neonates, rates of asymptomatic colonization can reach 70%, with colonization peaks occurring within the first months of life and up to 18 months (87).

More recently, prospective testing for *C. difficile* in children under the age of two years found that 33% of them were colonized overall (88). As a result of high colonization rates in infants under age of two years, and to avoid false positive due to asymptomatic colonization, this age group was excluded in our study.

C. difficile presentation in children is usually mild to moderate, and it is in most of cases missed, as it results in presentation similar to that seen in amoebiasis, which is treated with metronidazole as first choice antibiotic, and this is the case in *C. difficile*, resulting in infection resolution and the *C. difficile* infection will be missed. According to a study in Philippines *C. difficile* infection is widespread and might go unnoticed in areas where intestinal parasitism and amoebiasis are widespread.(89)

In our study toxigenic *C. difficile* strains were found among 10.6 % of the children group, and it was distributed in 14.8 % (n=4) of the first age group 2- < 5 yrs. and it was declining in the remaining groups, 10 % (n= 3) from the second age group (5- < 10), and it was in 7.1 % of the third age group . Our findings come parallel to findings of a recently published study in Qatar, that investigated *C. difficile* in children ages between 5- 14 years (90).

Major risk factors associated with *C. difficile* infection in children, were previous antibiotic exposure and hospitalization (90), in our study there were positive association between these factors and CDI, with P values 0.03 and 0.037, respectively. Cephalosporins (second and third generations), amoxicillin clavulanic acid, and ampicillin were most common antibiotic classes found in antibiotic use history, which considered as high-risk antibiotics for CDI.

As in prevalence group, the most common strain was A+B+, (66.7%), and no binary containing strains found in children group, although it was described in several reports in children population. This may be due to limited prevalence of binary positive isolates which was low in adult group, and overall prevalence of CDI in children group was lower than the adult group, as a result the binary toxin in children group is expected to be lower and may require lager sample size.

Concerning carrier status of non – toxigenic *C. difficile* strains, in the present study, it was found among 4.7 % of the children group. The colonization rates vary greatly by nation, age group, underlying disorders, and other concomitant circumstances, ranging from 0–10% to 30–80%. The colonization rate in a study that investigate colonization among older children was 6.6% with non-toxigenic strains .(91)

4.5 Conclusion

The overall prevalence of 15.7 % in prevalence group is alarming, especially when dealing with spore forming bacteria, that resist harsh condition and require special sterilization techniques, in addition to fertile back ground in our Palestinian community that had high percentage of antibiotic use, the main risk factor for infection. Antibiotic susceptibility patterns against vancomycin and metronidazole indicate that they are still effective, but resistance rates are warning especially with regard to metronidazole.

4.6 Recommendations

Adaptation of effective infection prevention control programs that target the control on transmission of infection through contact and isolation precautions will limit exposure to *C. difficile* spores and spread of infection, which is essential in prevention and management.

In addition to antibiotic stewardships to rationalize the use of antibiotics is the other arm of prevention, which found to be effective in reduction of CDI rates. Furthermore, raising awareness through health education that targets all categories , at the individual and institutional levels, about the ways of transmission of infection and strategies to prevent it.

We conclude our recommendations with the known proverb "An ounce of prevention is better than a pound of cure" .

4.7 Limitations

During our work in this research, there were several obstacles that faced us, including difficulty in sample collection, some hospitals refuse to collect samples for our research, and there was difficulty in availability of materials needed for this research. In addition to limited budget.

List of Abbreviations

Abbreviation	Meaning
C. difficile	*Clostridium difficle*
CDI	*C. difficile* infection
EIA	Enzyme immunoassay
GDH	glutamate dehydrogenase
MOH	Ministry of health
NAAT	Nucleic acid amplification technique
NNUH	An- najah national university hospital
NTC	Non – Toxigenic *C. difficile*
PCR	Polymerase chain reaction
PPI	Proton Pump Inhibitors

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Appendices

Appendix A

Tables of Study

Table A 1

PCR program

Temperature	Time	Cycles
94 °C	15 minutes	1
94 °C	45 seconds	35
50°C	45 seconds	35
72°C	1 minute	35
72°C	30 minutes	1
15 °C	Hold	

Table A 2

Result interpretation

Gene	Size
TcdA	629 bp
TcdB	410 bp
CdtA	221 bp
CdtB	262 bp
16S	1062 bp
GDH	158 bp

Table A 3

Multinomial regression

Variable	p- value in multinomial regression model
Antibiotic use	0.004
PPI use	0.03
Previous hospitalization	0.0010
Comorbidities	0.0014

Table A4

Prevalence cross table

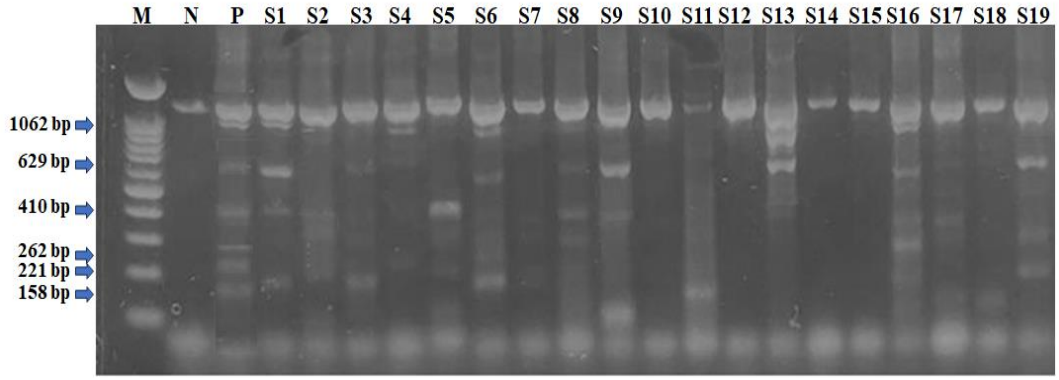
Result	Frequency	Percent
Negative	116	82.9%
Positive	22	15.7%
Carrier	2	1.4 %
Total	140	100.0

Appendix B

Figures of Study

Figure B 1

gel electrophoreses for random samples from comparison group



(M) Marker, DNA ladder, (N) Negative control (P) Positive control, (S1) sample 1: A+B+, Sample 2 (S2) A-B+, (S3):sample 3 A+B-, (s18) GDH + (carrier).

Figure B 2

Sub- sample 2 residency distribution by governates

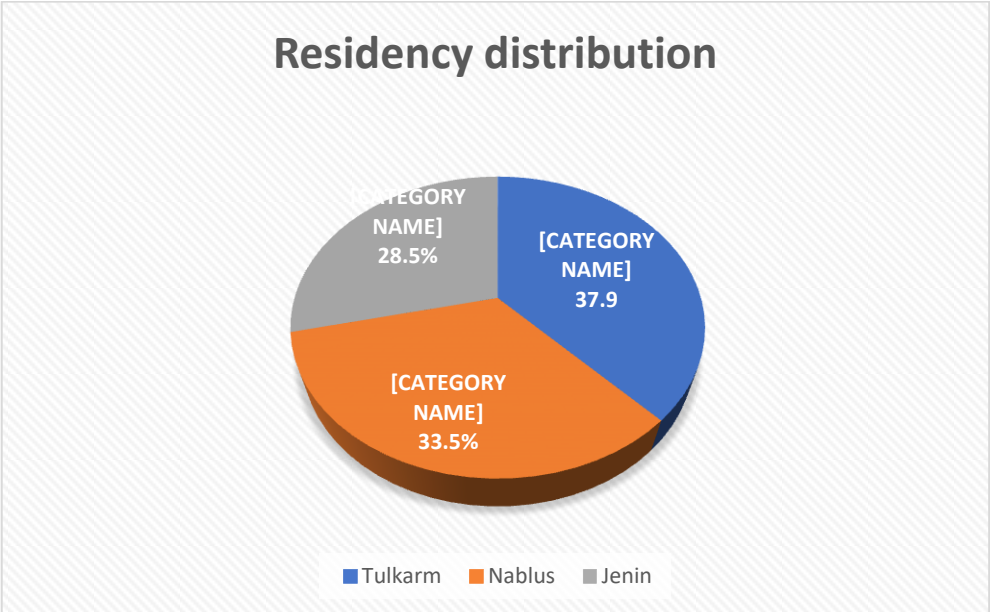


Figure B 3

Antibiotic classes associated with C.difficile infection sub- sample 2

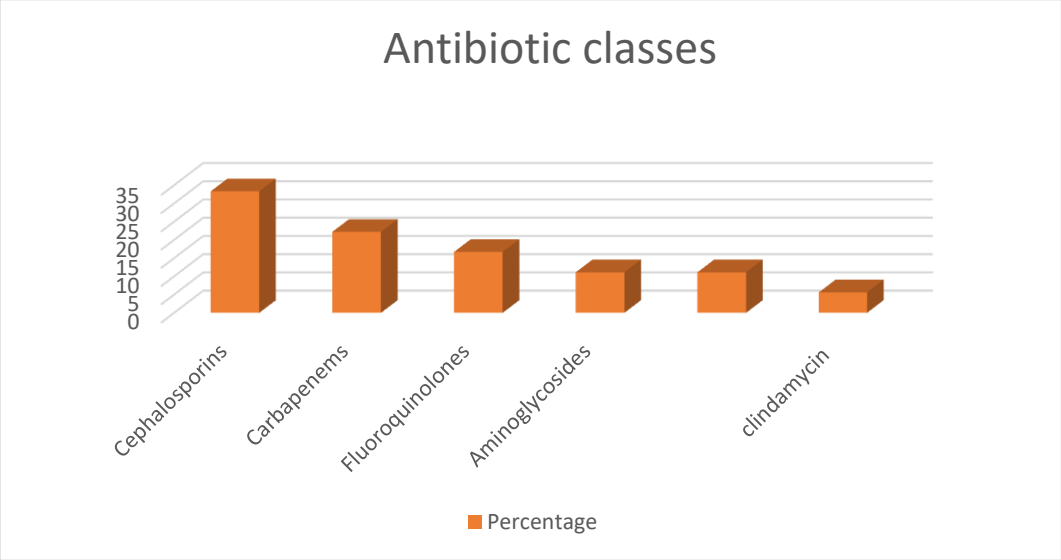


Figure B 4

gender distribution in sub-sample 3

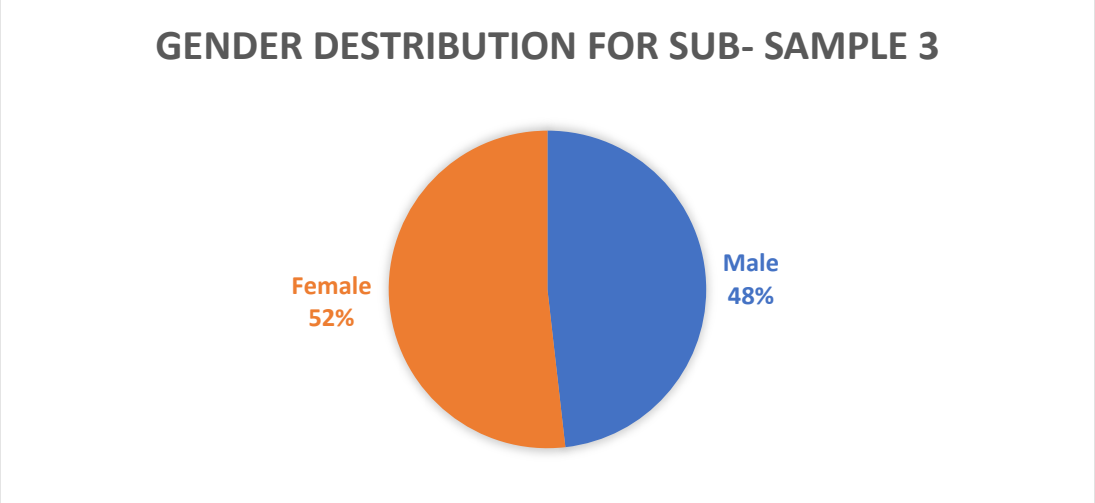


Figure B 5

Residency distribution, sub- sample 3

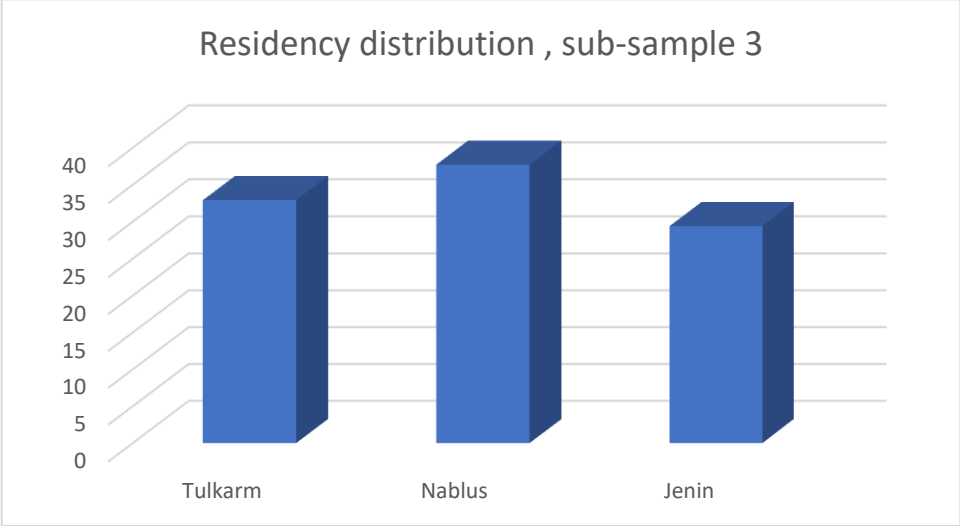
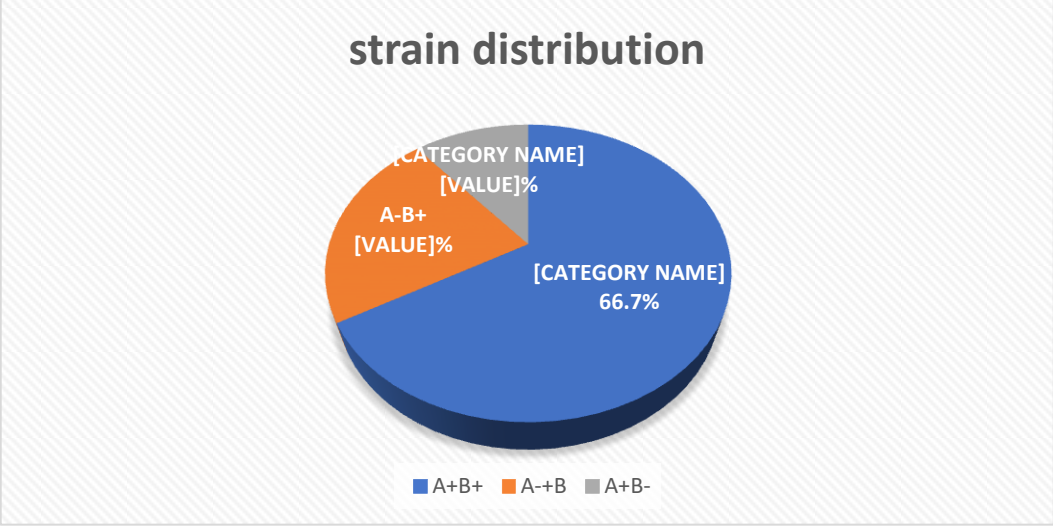


Figure B 6

Strain distribution, sub-sample 3



Appendix C

IRB approval

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. Feb. 2022/26

IRB Approval Letter

Title of Research:

Prevalence of Clostridium. difficile toxigenic strains and molecular characterization of antibiotic resistance patterns among Palestinians in west bank.

Submitted by:
Deema Khouli

Supervisor:
Walid Bshsa

Approved:
23nd Feb. 2022

Your Study Title **"Prevalence of Clostridium. difficile toxigenic strains and molecular characterization of antibiotic resistance patterns among Palestinians in west bank.** reviewed by An-Najah National University IRB committee and was approved on 23nd Feb. 2022.

Hasan Fitian, MD
IRB Committee Chairman

IRB

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix D

Ministry of health correspondence

An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ : 2022/5/30م

حضرة الدكتور عبد الله القواسمي المحترم
مدير عام التعليم الصحي / وزارة الصحة الفلسطينية

الموضوع: تسهيل مهمة الطالبة/ ديما راسم جميل خولي رقم تسجيل (12053656)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ،،،

الطالبة/ ديما راسم جميل خولي، رقم تسجيل 12053656، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

معدل انتشار الفصائل السامه من بكتيريا الكلوستريديوم ديفيسيل ووصف انماط مقاومة المضادات الحيويه
Prevalence of *Clostridium. difficile* toxigenic strains and characterization of antibiotic resistance patterns among Palestinians in North west bank

يرجى من حضرتكم تسهيل مهمتها في جمع البيانات والمعلومات حول معدل انتشار الفصائل السامة من بكتيريا كلوستريدوم ديفيسيل في عينات براز المرضى الذين يعانون من الإسهال وايضا بيانات المرضى المتعلقة بالتاريخ المرضي لهم، وذلك في المستشفيات الحكومية التالية: (مستشفى الشهيد ثابت ثابت الحكومي/ طولكرم، مستشفى رفيديا الجراحي/ نابلس، مستشفى جنين الحكومي والمستشفى الوطني الحكومي).

علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

مع وافر الاحترام ،،،

جامعة النجاح الوطنية
كلية الدراسات العليا

أ.د. وليد عويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 707، 7 هاتف:/2345115، 2345114، 2345113 (09)(972)* فاكسميل:2342907(09)(972)
Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115 هاتف داخلي (5) 3200
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Appendix E

An-najah national university hospital correspondence

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

An-Najah
National University
Faculty of Graduate Studies

التاريخ : 2022/5/17م

حضرة الدكتور كمال حجازي المحترم
مدير عام مستشفى النجاح الوطني الجامعي

الموضوع: تسهيل مهمة الطالبة/ ديما راسم جميل خولي رقم تسجيل (12053656)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ،،،

الطالبة/ ديما راسم جميل خولي، رقم تسجيل 12053656، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

معدل انتشار الفصائل السامه من بكتيريا الكلوستريديوم ديفيسيل ووصف انماط مقاومة المضادات الحيويه

Prevalence of *Clostridium. difficile* toxigenic strains and characterization of antibiotic resistance patterns among Palestinians in North west bank

يرجى من حضرتكم تسهيل مهمتها في جمع البيانات والمعلومات حول معدل انتشار الفصائل السامة من بكتيريا كلوستريدوم ديفيسيل في عينات براز المرضى الذين يعانون من الإسهال وايضا بيانات المرضى المتعلقة بالتاريخ المرضي لهم في مشفاكم الموقر.

علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

مع وافر الاحترام،،،

أ.د. وليد صويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 707 هاتف:/2345115، 2345114، 2345113 (09)(972)* فاكسميل:2342907(09)(972)
Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115 هاتف داخلي (5) 3200
* Facsimile 972 92342907 *www.najah.edu - email fgs@najah.edu

Appendix F

Specialized -Arab hospital correspondence

An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ : 2022/5/30م

مدير عام المستشفى العربي التخصصي المحترم
نابلس

الموضوع: تسهيل مهمة الطالبة/ ديما راسم جميل خولي رقم تسجيل (12053656)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ،،،

الطالبة/ ديما راسم جميل خولي، رقم تسجيل 12053656، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

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علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

مع وافر الاحترام،،،

أ.د. وليد صويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 7، 707 هاتف:/2345115، 2345114، 2345113 (09)(972)* فاكسميل:2342907(09)(972)
هاتف داخلي (5) 3200 Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115
* Facsimile 972 92342907 *www.najah.edu - email fgs@najah.edu

Appendix G

Data collection form

General characteristic:			
Hospital……	Clinic……		
Age ……..	Sex	• Male	• Female
Place of residence	…….		
Diagnosis (comorbidities)	……		
Clinical symptoms	1. Diarrhea	2. Fever > 38^{o}	3. Abdominal pain
Antibiotic use history / type :	…… ….		
PPI use history :	…… ….		
Hospitalization history	……		
Lab. Test Results			
- **WBC count:** - **Creatinine:**			

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

التوصيف الجزيئي للفصائل السامة من بكتيريا كلوستريديوم ديفيسيل وانماط حساسية المضادات الحيوية بين الفلسطينين في اجزاء من شمال الضفة الغربية

إعداد

ديما راسم خولي

إشراف

د. وليد الباشا

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في مكافحة وضبط العدوى، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2023

التوصيف الجزيئي للفصائل السامة من بكتيريا كلوستريديوم ديفيسيل وانماط حساسية المضادات الحيوية بين الفلسطينين في اجزاء من شمال الضفة الغربية

إعداد

ديما راسم خولي

إشراف

د. وليد الباشا

الملخص

المقدمة: تُعَرف بكتيريا كلوستريديوم ديفيسيل بأنّها عصويّة، مكونة للأبواغ، موجبة الجرام، وتسبب مرضًا معديًا (عدوى كلوستيدوم ديفسيل)؛ حيث تنتقل هذه العدوى عن طريق الفم، وتنبت الأبواغ في المعدة، وتنتج سمومًا ضارة (أ، ب، السم الثنائي) التي تعدُّ، بدورها، المسؤولة عن الأعراض السريرية للعدوى؛ فالإسهال الالتهابي – وهو العرض الرئيس بين المرضى المصابين– يمكن أن يكون معتدلًا ومحدودًا ذاتيًا، ولكن يمكن أن يكون شديدًا أيضًا، بنحو يؤدي إلى مضاعفات خطيرة، مثل تضخم القولون السام والتهاب القولون الغشائي الكاذب. ويعد الاستخدام غير الرشيد للمضادات الحيوية واسعة النطاق، والعمر فوق 65 عامًا، والإقامة الطويلة في المستشفى، وحالة نقص المناعة، بالإضافة الي استخدام مثبطات مضخات البرتونات أهم عوامل الخطورة المرتبطة بقابلية العدوى لدى الأشخاص. **الأهداف:** هدفت هذه الدراسة إلى التوصيف الجزيئي لسلالات كلوستريديوم ديفيسيل المنتجة للسموم، وتحديد معدل انتشارها، وعوامل الخطر المرتبطة بها بين الفلسطينيين في الجزء الشمالي من الضفة الغربية، بالإضافة إلى حساسية هذه البكتيريا لخيارات الخط الأول من المضادات الحيوية، وأخيرًا تسليط الضوء على أثر هذه العدوى في الأطفال. **المنهجية البحثية:** تم اختيار تصميم دراسة مقطعية، وجمع عينات الإسهال للبراز من المرضى في المستشفيات الحكومية، ثُمَّ تمّ إجراء التوصيف الجزيئي عن طريق تفاعل البوليميراز المتسلسل، ومن بعدُ، جرى فصل عينات تفاعل البوليميراز باستخدام تقنية الفصل الكهربائي، متبوعا بعزل المستعمرات البكتيرية

باستخدام وسط غذائي متخصص لهذا النوع من البكتيريا فقط، ثم جرى عمل فحص حساسية المضادات الحيوية باستخدام طريقة الانتشار حول قرص المضاد الحيوي. **النتائج:** كان انتشار البكتيريا في مجموعة البالغين 15.7 %، ونسبة الإصابة 5.8 لكل 10000 مريض إسهال، وكان الانتشار في مجموعة الأطفال 10.6%. وأظهرت الدراسة أنّ عوامل الخطر في الانتشار تتمثّل في استخدام المضادات الحيوية، واستخدام مثبطات مضخة البروتون، ووجود زيارات سابقة للمستشفيات والأمراض المصاحبة، وتمثَّلت عوامل الخطر لدى مجموعة الأطفال في استخدام المضادات الحيوية، وتاريخ زيارة المستشفيات. كانت البكتيريا المعزولة حساسة بنسبة 96٪ و89٪ ضد فانكومايسين، وميترونيدازول على التوالي. **الخاتمة:** يعتبر الانتشار 15.7 علامة تحذير، خاصة عند التعامل مع البكتيريا المكونة للأبواغ، وفي مجموعة الأطفال كانت معظم الحالات مشخصة بالخطأ بالأميبا؛ لأنها تؤدي إلى عوارض مماثلة، وتعالج بنفس المضاد الحيوي. وللسيطرة على انتشار عدوى كلوستيديوم ديفسيل؛ توصي الدراسة بضرورة زيادة الوعي حول ممارسات الوقاية من العدوى في المستشفى والمجتمع، بالإضافة إلى الحاجة لبرامج الإشراف على المضادات الحيوية.

الكلمات المفتاحية: كلوستريديوم ديفيسيل، الاسهال الالتهابي، السم أ، السم ب، السم الثنائي.