

Antimicrobial Effects of Three-Types of Metronidazole Drug on *Salmonella Enterica* and *Vibrio Fluvialis* Isolated from Thi-Qar Province

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ABSTRACT

The study examined the effect of Metronidazole on *Salmonella enterica* and *vibrio fluvialis* isolates, the test was carried out by using a Metronidazole from three companies (French company, Iraqi and Egyptian), the two isolates were taken from diarrheal patients, some were resistant, others were sensitive and some areas of inhibition were shown using treatment from the three companies, the effectiveness of the treatment of the French company. This study has examined the effect of three different type of Metronidazole from three companies (France, Egypt and Iraqi) on some Enterobacteriaceae. The results indicated the dominance of Metronidazole under France companies on other companies, as it effects on *Salmonella enterica* and *Vibrio fluvialis* at two concentrations, and it had an inhibitory effect on *Vibrio fluvialis* at concentrations (5 mg/ml), while Metronidazole under Egypt company had only a inhibitory effect on *vibrio fluvialis* at two concentrations (10 and 15 mg/ml) and Metronidazole under Iraqi company were effective on *vibrio fluvialis* at two concentrations also (10 and 15 mg/ml). These results indicate that Metronidazole under France company is the best antibiotic to be used in case of diarrhea that caused by *Vibrio fluvialis* and *Salmonella enterica*

Keywords: *Salmonella enterica*, *Vibrio fluvialis*, Metronidazole.

1 INTRODUCTION

Salmonella enterica is one of the most commonly detected, in terms of both numbers of human infections and severe disease; the widespread of *S. enterica* in humans and animals worldwide have always been a major public health concern [1]. *V. fluvialis* is considered one of the foodborne pathogenic bacteria and has been implicated in outbreaks and sporadic cases of diarrhea [2]. Metronidazole, marketed under the brand name Metronidazole, is an antibiotic and antiprotozoal medication [3]. It is used either alone or with other antibiotics to treat pelvic inflammatory disease, endocarditis, and bacterial vaginitis. It is effective for dracunculiasis, giardiasis, trichomoniasis, and amebiasis. It is an option for a first episode of mild-to-moderate *Clostridium difficile* colitis if vancomycin or fidaxomicin is unavailable [4]. Metronida-

zole is available by mouth, as a cream, and intravenously. Common side effects include nausea, a metallic taste, loss of appetite, and headaches [3]. Occasionally seizures or allergies to the medication may occur. Some state that metronidazole should not be used in early pregnancy while others state doses for trichomoniasis are safe. It should not be used when breast feeding [5]. Metronidazole began to be commercially used in 1960 in France [6]. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system [7]. It is available in most areas of the world [8]. Aim of study is Testing pharmacological effectiveness of metronidazole (Metronidazole) from several companies against bacterial intestinal by: Identification of some genera of bacterial intestinal isolated from diarrheal patients and examination of the isolated to metronidazole

susceptibility and resistant.

2 MATERIALS AND METHODS

2.1 Culture media

The culture media shown in table below have been used for isolation and identification of bacteria isolation are in Tabel 1.

Table 1. The culture media used in this research.

No.	Culture media	Company	Origin
1	Blood agar	TITAN BIOTECH LTD	India
2	Muller henton agar	TITAN BIOTECH LTD	India
3	Brain Heart infusion agar	TITAN BIOTECH LTD	India
4	MacConkey agar	TITAN BIOTECH LTD	India

2.2 Antimicrobial METRONIDAZOLE

The antimicrobial disks that shown in table were used for detection the susceptibility of bacteria isolate to four selective antibiotics in Table 2 and Fig.1.

Table 2. Antimicrobial Metronidazole.

NO.	Antibiotics	Common name	Company	Origin
1	Metronidazole	Metronidazole	SANOFI	France
2	Metronidazole	Metronidazole	SANOFI	Egypt
3	Metronidazole	Safagyl	SAFA	Iraq



Fig. 1. Antimicrobial Metronidazole.

2.3 Methods

2.3.1 Collecting of samples

Two isolates from people with diarrhea were obtained from the laboratories of the Department of biology Sciences in the college of Science, Thi-Qar University (*Vibrio fluvialis* and *Salmonella enterica*). After definitive diagnosis of the isolates were reactivated by cultured on blood agar

and MacConkey agar and then incubated at 37 °C for 24 hours before Antimicrobial sensitivity test.



Fig. 2. *Vibrio fluvialis* on Blood agar

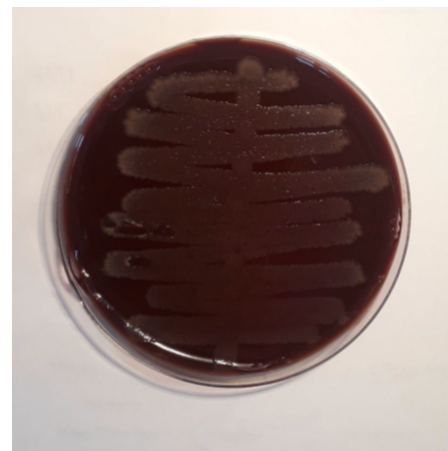


Fig. 3. *Salmonella enterica* on Blood agar.

2.4 Antimicrobial sensitivity test

A method of [9] was used to estimate the metronidazole sensitivity of both isolate bacteria *Vibrio fluvialis* and *Salmonella enterica* which isolated from patients with diarrhea. The culture media (Muller Hinton agar) were prepared according to the instruction of the manufacture company stated on the container and it was autoclaved at 121C for 15 min and then inoculated the isolates in Muller Hinton agar. The antibiotic drugs are applied to the culture media in three concentration (5 mg/ml, 10 mg/ml and 15 mg/ml) by making a hole and incubated at 37C for 24 hours. The effectiveness of a particular antibiotic is shown by the presence of growth - inhibitory zones, these zones of constraint (ZOIs) appear as clear areas surrounding the holes from which the substances of antimicrobial activity diffused, diameter of the zones can be measured by Electronic ruler in Millimeters

3 RESULTS AND DISCUSSION

The study examined the effect of Metronidazole on *Salmonella enterica* and *vibrio fluvialis* isolates, the test was carried out by using a Metronidazole from three companies (French company, Iraqi and Egyptian). One of the most important bacterial enteric pathogens is *Salmonella enterica*, it is a bacterium that cause Salmonellosis, and attendant public health problem [10]. *Salmonella enterica* regardless as important pathogen in people of all ages but the severe invasive disease in infants, elderly persons, and immunocompromised persons [11]. *V. fluvialis* is one of the emerging foodborne pathogens all over the world. The distribution of virulence factors and molecular epidemiological features of this pathogen remain mostly unknown. Among the foodborne infections in the United States, there has been a considerable increase (43%) in the *Vibrio*-mediated infections till 2012 compared with the rates reported during 2006–2008 [12]. Several recent publications indicate the epidemiological importance of *V. fluvialis* [13]. Also, [14] found that 3-million children were killed each year in the developing countries due to diarrhea. Metronidazole is a drug used to treat a wide variety of infections caused by certain types of germ (anaerobic bacteria) and types of micro-organisms called protozoa. These types of organisms often cause infections in areas of the body such as the gums, pelvic cavity and tummy (stomach or intestines) because they do not need oxygen to grow and multiply [15]. Metronidazole is commonly prescribed to treat an infection called bacterial vaginosis. It is also prescribed before gynecological surgery and surgery on the intestines to prevent infection from developing. Metronidazole is active against a variety of protozoa and bacteria. It enters the cell as a prodrug by passive diffusion and is activated in either the cytoplasm of the bacteria or specific organelles in the protozoa [16]. This study has examined the effect of three different type of Metronidazole from three companies (France, Egypt and Iraqi) on some Enterobacteriaceae. The results indicated the dominance of Metronidazole under France companies on other companies, as it effects on *Salmonella enterica* and *Vibrio fluvialis* at two concentrations, and it had an inhibitory effect on *Vibrio fluvialis* at concentrations (5 mg/ml), while Metronidazole under Egypt company had only a inhibitory effect on *Vibrio fluvialis* at two concentrations (10 and 15 mg/ml) and Metronidazole under Iraqi company were effective on *Vibrio fluvialis* at two concentrations also (10 and 15 mg/ml).These results indicate that Metronidazole under France company is the best antibiotic to be used in case of diarrhea that caused by *Vibrio fluvialis* and *Salmonella enterica* as in Tables (3,4,5) and Figs.(4,5,6); *Salmonella enterica* was sensitive to the France Metronidazole with concentration (15 mg/ml and 5 mg/ml) But were resistant at a concentration of (10 mg/ml). While the *vibrio fluvialis* were sensitive at concentration (10mg/ml and 15mg/ml) But its inhibitor was at concentration (5 mg/ml).

Table 3. Metronidazole in company SANOFI, France.

Type of bacteria	Concentration		
	5 mg/ml	10 mg/ml	15 mg/ml
<i>Salmonella enterica</i>	S	R	S
<i>Vibrio fluvialis</i>	I	S	S

Salmonella enterica was resistant to the Egypt Metronidazole with concentration (5 mg/ml and 10 mg/ml and 15 mg/ml). While the *Vibrio fluvialis* were resistant at concentration (5mg/ml) But its inhibitor was at concentration (10 mg/ml and 15mg/ml).

Table 4. Metronidazole in company SANOFI, Egypt.

Type of bacteria	Concentration		
	5 mg/ml	10 mg/ml	15 mg/ml
<i>Salmonella enterica</i>	R	R	R
<i>Vibrio fluvialis</i>	R	I	I

Salmonella enterica was resistant to the Iraq Metronidazole with concentration (5 mg/ml, 10 mg/ml and 15 mg/ml). While the *vibrio fluvialis* were resistant at concentration (5mg/ml) But its sensitive was at concentration (10 mg/ml and 15mg/ml).

Table 5. Metronidazole in company SAFA, Iraq.

Type of bacteria	Concentration		
	5 mg/ml	10 mg/ml	15 mg/ml
<i>Salmonella enterica</i>	R	R	R
<i>Vibrio fluvialis</i>	R	S	S

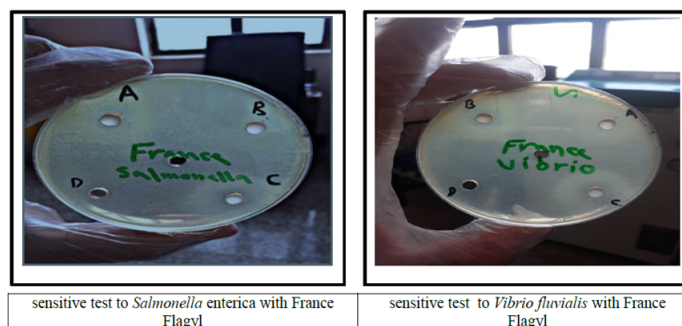


Fig. 4. Sensitive test with France Metronidazole.

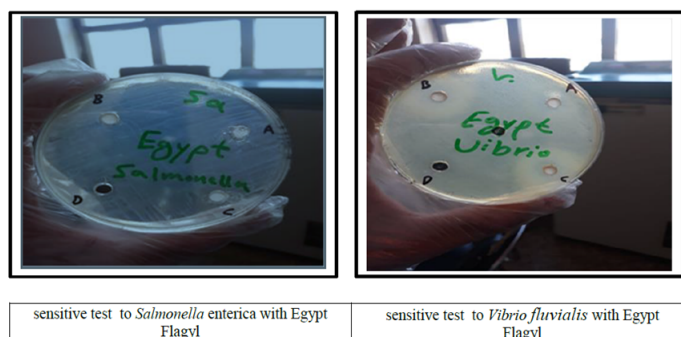


Fig. 5. Sensitive test with Egypt Metronidazole.

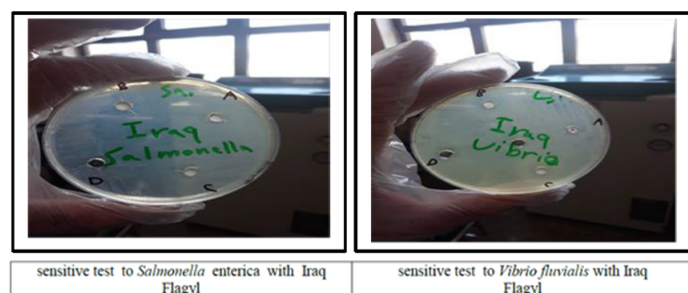


Fig. 6. Sensitive test with Iraq Metronidazole.

Whereas drug-resistant cells are deficient in drug activation. The metronidazole molecule is converted to a short-lived nitroso free radical by intracellular reduction, which includes the transfer of an electron to the nitro group of the drug. This form of the drug is cytotoxic and can interact with the DNA molecule. The actual mechanism of action has not yet been fully elucidated but includes the inhibition of DNA synthesis and DNA damage by oxidation, causing single-strand and double-strand breaks that lead to DNA degradation and cell death. The activated reduced metronidazole molecule binds nonspecifically to bacterial DNA, inactivating the organism's DNA and enzymes and leading to a high level of DNA breakage, with immediate action of the drug but no cell lysis [17]. Aerobic cells lack electron transport proteins with sufficient negative redox potential; therefore, the drug is active against only bacteria with anaerobic metabolisms. The current study used a variety of Metronidazole (Iraq, France and Egypt company) to test their effectiveness on some species of Enterobacteriaceae causing diarrhea. The study indicated that *Salmonella enterica* bacteria was sensitive to the France Metronidazole with concentration (5 mg/ml and 15 mg/ml) only, but were resistant to the France Metronidazole at a concentration of (10 mg/ml), Egypt Metronidazole with concentration (5 mg/ml and 10 mg/ml and 15 mg/ml) and Iraq Metronidazole with concentration (5 mg/ml, 10 mg/ml and 15 mg/ml). While the *Vibrio fluvialis* were sensitive to France Metronidazole at concentration (10mg/ml and 15mg/ml), Iraq Metronidazole at concentration (10 mg/ml and 15mg/ml). But its inhibitor by

France Metronidazole at concentration (5 mg/ml), Egypt Metronidazole at concentration (10 mg/ml and 15mg/ml). While resistant to the concentration (5mg/ml) and Iraq Metronidazole at concentration (5mg/ml). The uncontrolled using of antibiotic in animals' food treatment recent surveys with molecular techniques provide firm evidence indicating that the use of antimicrobial agents in animals' food contributes to the development of antimicrobial-resistant *Salmonella* that cause infections in humans [18]. During the last decades worldwide, the uncontrolled use of antimicrobials for growth promotion, treating human patients, veterinary field and prevention in conventional food production has led to the development of antibiotic resistance in *Salmonella* [19]. The prophylactic use of antibiotics in food animals is a major contributing factor for the increase in resistance to antibiotics [20].

In study by [21] appeared several large and small plasmids were extracted from *Salmonella* isolates out of 91.7% of this isolate carried one or more plasmids; these plasmids may be responsible about the resistance to antibiotic. This research demonstrated that the horizontal transfer of antibiotic resistance plasmids can occur among *Salmonella* isolates via conjugation and also revealed the emergence of multidrug-resistant *Salmonella* isolates as a significant health problem; this consistent with [22] and [23]. So that must Don't use any antibiotics without consultation the doctor and Increase research and studies on antibiotics manufactured by different companies in local pharmacies to know their side effects and their therapeutic effectiveness. Worldwide, Hanan et al., (2021) reported during the last decades, the uncontrolled using of antimicrobial agents for growth promotion, treating patient, veterinary fields and prevention in conventional food production have led to the event of antibiotic resistance in *Salmonella* [24].

Conflict of Interest: The authors declare no conflict of interest.

Financing: The study was performed without external funding.

Ethical consideration: The study was approved by University of Thi-Qar, Thi-Qar, Iraq.

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