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RESPONSE OF SOME MICROORGANISMS TO THERAPEUTIC (NEWLY EFFERVESCENT CIPROFLOXACIN AND CONVENTIONAL BRANDS)

***Ahmed M. A. Msaad**

Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Taif University, KSA.

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***Corresponding Author**
Dr. Ahmed M. A. Msaad
Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Taif University, KSA.

ABSTRACT

Typhoid is an epidemic disease in Sudan and causes morbidity for many peoples especially in tropical countries.[1] This research aimed to study the response of (*Salmonell typhi* and *Salmonella para typhi*) to the ciprofloxacin HCl (effervescent formulae ciprofloxacin and conventional five brands). It is a drug of choice used as an alternative to chloramphincol.[2] Then the microbiological sensitivity test was carried out against *(Salmonella typhi, Salmonella para typhi*) to detect the response of each one and comparison was held between conventional and unconventional tablets.[3] The results were found that the response of Salmonella typhi is less than Salmonella para typhi, this may be due to genetic factor that found in Salmonella typhi (which make it produce more poly saccharide rather than Salmonella para typhi).[4][19] Interestingly from this research the inhibition zones of effervescent tablets are greater than conventional tablets that may be an indication for more activity and may have quicker using response or action.[5][19]

KEYWORDS: *Salmonella typhi, Salmonella para typhi,* Effervescent ciprofloxacin HCl, response, Activity.

INTRODUCTION

Salmonellae are gram-negative motile bacilli. The genus *Salmonella,* which belongs to the family Enterobacteriaceae, was named after Daniel E. Salmon, an American veterinarian who first isolated *Salmonella choleraesuis* from pigs with hog cholera in 1884.[4]

As with the closely related bacterium *Escherichia coli*, salmonellae are potential enteric pathogens and a leading cause of bacterial foodborne illness. In addition, *Salmonella* species have been implicated in a spectrum of other diseases, including enteric or typhoid fever (primarily *Salmonella typhi* and *Salmonella paratyphi*), bacteremia, endovascular infections, focal infections (eg, osteomyelitis), and enterocolitis (typically *Salmonella typhimurium, Salmonella enteritidis*, and *Salmonella heidelberg*).

Salmonellae can be isolated in the microbiology laboratory using numerous low-selective media (MacConkey agar, deoxycholate agar), intermediate-selective media (*Salmonella-Shigella* [SS] agar, Hektoen [HE] agar), and highly selective media (selenite agar with brilliant green). Salmonellae are oxidase-negative and predominantly lactose-negative. Fever than 1% of nontyphoidal *Salmonella* isolates are lactose-positive (pink on MacConkey agar), but most produce hydrogen sulfide, which is detectable on HE or SS agar. As facultative anaerobes, they grow well both in bottles of standard automated systems for blood cultures and on culture media routinely used for urine, tissue, and respiratory cultures.[6] Individual isolates can then be distinguished with serogrouping, pulsed-field gel electrophoresis, and bacteriophage serotyping techniques.

Nomenclature and classification

The nomenclature and classification of *Salmonella* species have been changed and restructured multiple times. Traditionally, *Salmonella* species were named in accordance with the Kaufmann-White typing system, defined by different combinations of somatic O, surface Vi, and flagellar H antigens. In 2005, *Salmonella enterica* finally gained official approval as the type species of the genus *Salmonella*. The genus *Salmonella* also contains the species *Salmonella bongori* and *Salmonella subterranean*, which was recognized in 2005.[7]

Currently, *Salmonella* species have the serologically defined names appended as serovars or serotypes. For instance, the current nomenclature of *S typhi* is *S enterica* serovar Typhi. *S enterica* is preferred over confusing name *S choleraesuis*, which is also the name of a commonly isolated serotype.[8] To date, more than 2500 serovars of *S enterica* have been described. Certain serovars are host-restricted, while others have a broad host range.[9]

Pathophysiology

The transmission of salmonellae to a susceptible host usually occurs via consumption of contaminated foods. The most common sources of salmonellae include beef, poultry, and

eggs. In addition, human-to-human and animal-to-human transmissions can occur. For example, amphibian and reptile exposures are associated with approximately 74,000 *Salmonella* infections annually in the United States. *Salmonellosis* outbreaks have also been associated with handling chicks, ducklings, kittens, and hedgehogs.[10, 11, 12, 13, 14, 15] Recently, a study of 28 cases of *Styphimurium* identified pet rodents as a previously unrecognized source of human *Salmonella* infection.[16]

Ciprofloxacin HCl

Quinolone antibacterial drugs have been in use since 1964, when nalidixic acid was released. Oxolinic and cinoxacin were introduced somewhat later. These drugs have fallen into disuse because of their limited antibacterial spectra, and resistance to them rapidly develops.

The introduction of 6-fluoro and 7-(1-piperazinyl) group expanded the spectrum, increased potency and appears to have prevented the development of plasmid-mediated resistance.(figure 1 and 2).

The fluoroquinolones are bacteriostatic at low and bactericidal at high concentrations. Use as alternative to chloramphincol due to the high risk of chloramphincol so it consider a drug of choice for enteric fever.[17]

Mechanisms of Action

The drugs inhibit DNA gyrase (topoisomerase II), which results in abnormal linkage between opened DNA and the gyrase. Negative supercoiling (absent in mammalian nuclei) is impaired, so protein synthesis is prevented. [17]

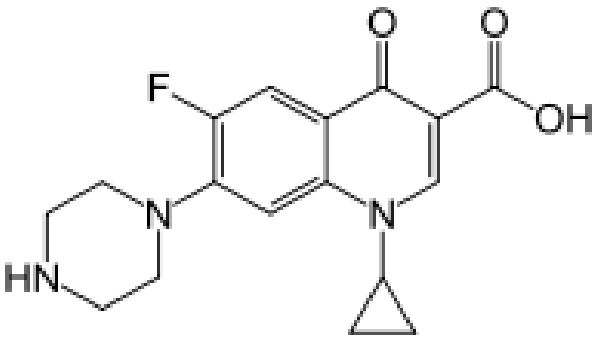

Figure 1: Structure of Ciprofloxacin.

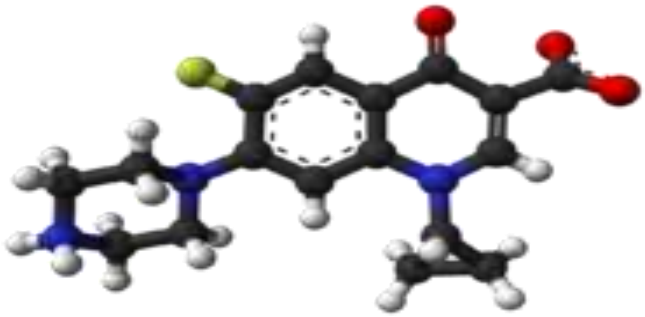

Figure 2: 3D Structure of Ciprofloxacin.

Microbiological Sensitivity Test

Antimicrobial susceptibility tests measure the ability of an antibiotic or other anti microbial agents under suitable conditions to inhibit bacterial growth in vitro for evaluating the safety and effectiveness of antibiotic products, several types of antimicrobial susceptibility (sensitivity) tests are done to detect the response of *Salmonella typhi* and *Salmonella para* typhi to drug ciprofloxacin.[3]

Ciprofloxacin Sensitivity Test using Disc diffusion Kirby-Bauer

Sensitivity Test

For each test and standard 1mg is taken and dissolved in 10 ml distilled water then 1ml was taken from it and dissolved in other10 ml distilled water.[3]

Antibiotic disc preparation

- Filter paper was cut into small disks of about 4 mm in diameter then it enclosed in a sealed container and sterilized in oven.
- Halve number of the disks impregnated with ciprofloxacin test suspension and the others with standard suspension then the disks are dried in oven at $60C^0$ for 20 minutes (serial dilution was made to obtain concentration 10µg/ml as follow: 1mg was dissolve in 10ml and then 1ml was taken and dissolve in another 10 ml).
- *Salmonella typhi*
- *Salmonella para typhi*

Inoculums preparation is the most important step in any susceptibility test. Inocula are prepared directly by inoculating colonies grown overnight on an agar plate, into broth media. Then the numbers of bacteria tested was standardized using McFarland turbidity standards.[3]

McFarland turbidity standards: The McFarland 0.5 standard is used, which contains 99.5 ml of 1% sulfuric acid and 0.5 ml of 1.175% barium chloride, this solution is dispensed into tubes comparable to those used for inoculums preparation.

The McFarland 0.5 standard provides turbidity comparable to that of a bacterial suspension containing 1.5×10^{8}CFU/ml.[3]

Inoculation and incubation

After preparation of standard inoculums suspension, a sterile cotton swab is dipped into the suspension, pressed to remove excess liquid, and then swabbed evenly across the surface of a Mueller Hinton agar plate (plates of 9mm are used). (Each inoculum suspension was inoculated into three media labeled test (T), standard (S) and control(C).

- Within 15 minutes of inoculation, the individual ciprofloxacin disks (one disc per plate) are applied to the agar media with a forceps and gently pressed to ensure contact with the agar.[3]
- The ciprofloxacin Test disks are applied in the plates labeled (T).
- The ciprofloxacin Standard disks are applied in the plates labeled(S). While other plate's labeled (c) without antibiotic disks were used to control growth.
- Within 15 minutes of disks placement, plates are inverted and placed in a 37 C^{0} for 18 hours.[3]
- After incubation the plates were examined, to make certain the test organisms has grown satisfactory, the diameter of each inhibition zone is measured using ruler or calipers.[3]
- Once zone measurements have been made, the millimeter reading for each brand and effervescent formula are compared with that specified in the interpretive tables of the NCCLS documents.

RESULTS

The response of both microorganisms against ciprofloxacin HCl (effervescent ciprofloxacin and conventional five brands with pure ciprofloxacin HCl) as shown in (figure 3 and figure 4).

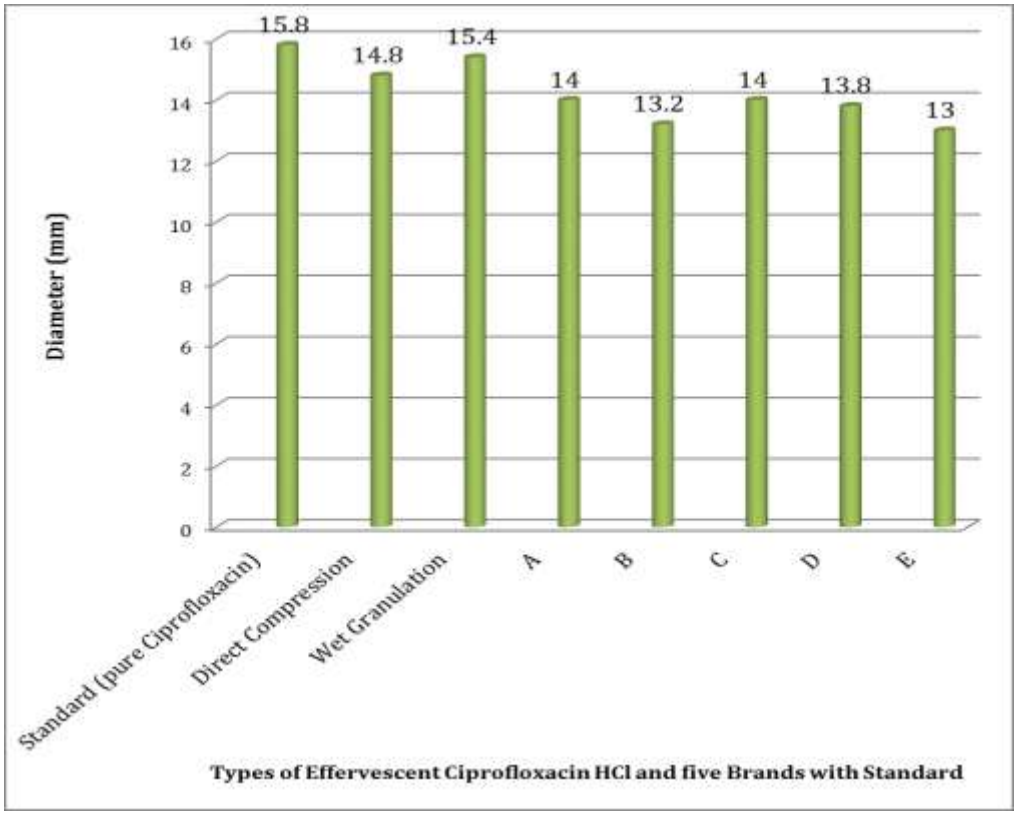

Figure 3: Response of *Salmonella typhi* to Ciprofloxacin HCl (Effervescent Ciprofloxacin and Conventional Five Brands with Pure Ciprofloxacin HCl).[19]

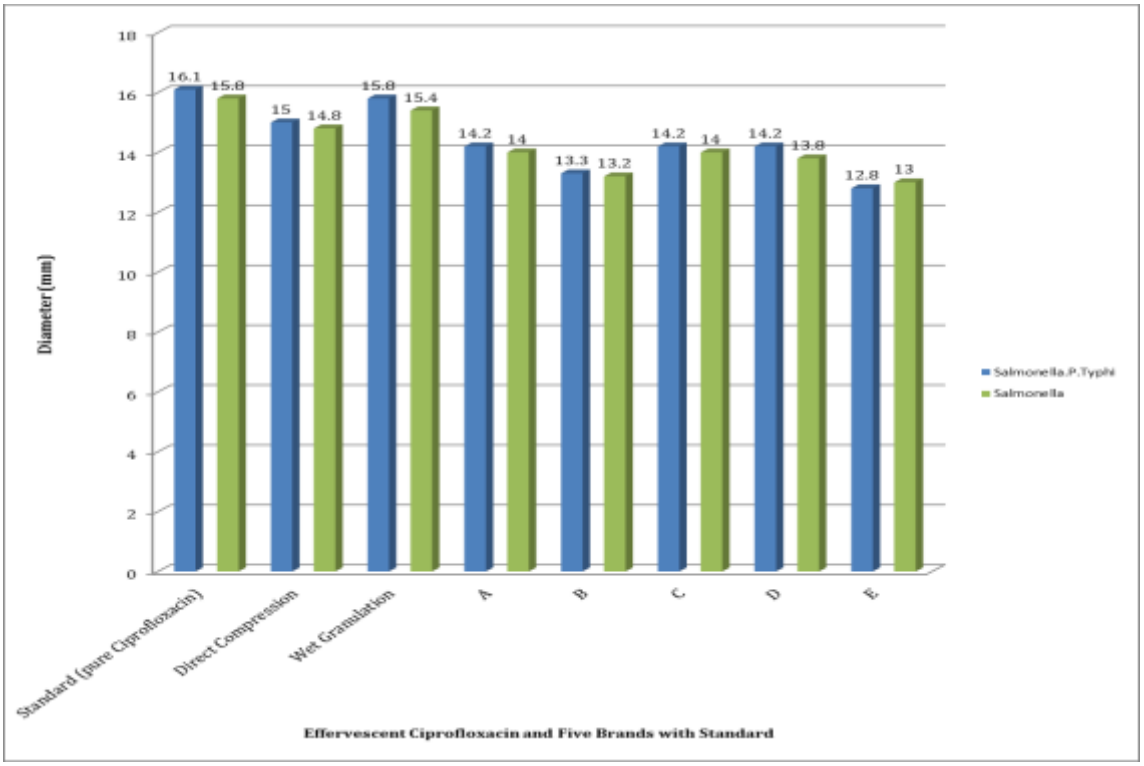

Figure 4: Comparison the Effect of all tablets and and standard on *Salmonella typhi* and *Salmonella P. typhi* by Diameter of Inhibition Zone.[19]

DISCUSSION

The disk was used in concentration 10µg according to (Oxoid Ltd, England) to give the require effect. For Accuracy and precession of the result standard *Salmonella typhi* and *Salmonella para typhi* according to the national committee for clinical laboratory standards (NCCTS).[3]

The diameter of each inhibition zone was found after measuring, using a ruler and calibers for each brand as shown in (figure 3 and 4). It is clear that arrangement of inhibition zone from microbiological results of zones of inhibition brand A > brand C > brand D > brand B >E. All brands are active against selected bacteria,[19] this result agreed with study by Mughal et al, (2009) and Asghar et al, (2009).

The effect of effervescent ciprofloxacin HCl is clear that higher than conventional five brands this might solve the problem of resistance microorganisms.[3] [19]

On comparing tablets formulated as effervescent ones (wet granulation) inhibition zones were found higher than the direct compression ones. This might be due to more distribution of the active ingredient by mixing.[5][19]

The response of *Salmonella para typhi* is higher sensitive than *Salmonella typhi* that clear in figure 4, this may be due to variation in genome of salmonella prar typhi comparing to *Salmonella typhi* that lead *Salmonella typhi* to produce more polysaccharide and Vi antigen which responsible for pathogenic effect and resistance.[18]

Figure 5: Inhibition Zone of *Salmonella typhi* and *Salmonella para typhi.*

CONCLUSION

- The higher sensitivity of *Salmonella para ty*phi comparing to *Salmonell typhi* may be due to the genetic factor that make *Salmonella typhi* produce more polysaccharide which might be responsible for resistance.[19]
- Effervescent ciprofloxacin tablets showed higher inhibition zones compared to conventional ciprofloxacin HCl brands, and this may lead to quicker response.
- The drug should be restricted for the treatment of the enteric fever, and don not use irrationally.
- Effervescent tablet was selected due to its convenient, easy to use, more effective and increase patient acceptability and palatability.

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