

Research Article

Efficacy of Chloroquine against *Escherichia Coli* and *Proteus vulgaris*: An in vitro Study

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Abstract: Chloroquine, a 4-aminoquinoline is an effective antimalarial drug and is also used as a systemic amoebicide in the treatment of hepatic amoebiasis (extra intestinal site). The objective of this study is to establish the efficacy of Chloroquine against common pathogenic bacteria such as *Escherichia coli*, and *proteus*. A stock solution containing 64 mg Chloroquine phosphate/mL was prepared in distilled water, serial dilution was prepared to obtain 5 mL solution, each having a different concentration of the drug. Whatman No.1 filter paper was used to prepare discs of 6mm diameter. The discs were sterilized and each dilution of the drug was added on the disc at a volume of 10µL per disc. Final concentration of Chloroquine phosphate per disc was 64, 53, 42, 30 and 21µg. Discs were dried and stored at 4°C. Disc of amoxicillin-clavulanic acid combination at strength of 30µg was used as a control. Total 100 isolates of bacteria isolates from clinical samples was used in the study. Antimicrobial susceptibility test of these isolates was performed by using Kirby-Bauer's method. It was observed that *E. coli* and *Proteus* were susceptible to chloroquine at higher strengths. Interpretation and chloroquine certainly shows certain degree of antimicrobial efficacy. In-vitro studies can be a stepping stone for further investigations in-vivo.

Keywords: Chloroquine, Antimicrobial Susceptibility Test, Efficacy, *E. coli*, *Proteus*

INTRODUCTION

Chloroquine is one of a large series of 4-aminoquinolines that was investigated in connection with antimalarial research in the United States during the World War II. In 1934 at the Elberfeld Laboratories of the Bayer I.G. Farbenindustrie A.G., H. Andersag synthesized a salt 2,3-dihydroxybenzoic acid, called Resochin, being the Resorcinate of a 4-amino Chinolin [1]. Although the 4-aminoquinolones had previously been described as potential antimalarials by the Russians investigators, serious attention was not paid to the group until the French reported that 3-methyl -7-chloro- 4- (4-diethylamino-1-methyl butylamino) quinolines (SN-6911; sontochin, sontoquin) was well tolerated and had high activity in human malaras. Beginning in 1943, a large number of these compounds was synthesized and tested for activity in avian malaria and for toxicity in mammals; ten of the series were examined in humans with experimentally induced malaras. Of these, chloroquine proved most promising

Chloroquine is effective against *P. vivax*, *P. ovale*, *P. malariae* and sensitive strain of *P. falciparum*. It exerts activity against gametocytes of the first three plasmodial species except *P. falciparum*. It has no activity against the latent tissue form of vivax and

ovale. Chloroquine or its analogs are also used for therapy of conditions other than malaria [2] such as hepatic amoebiasis. Chloroquine and hydroxychloroquine have been used as secondary drugs to treat a variety of chronic diseases, because both of them concentrate in lysosomes and have anti-inflammatory properties [3]. High doses of these compounds, often together with other agents are clinical efficient in rheumatoid arthritis, systemic lupus erythematosus, discoid lupus, sarcoidosis, and photosensitivity diseases such as porphyria cutanea tarda and severe polymorphous light eruption [4].

Chloroquine is not recommended for treating epilepsy or myasthenia gravis. It should be used cautiously in the presence of hepatic disease or severe gastrointestinal, neurological, or blood disorders [5].

Escherichia coli abbreviated as *E. coli* belonging to Enterobacteriaceae, was discovered by pediatrician and bacteriologist Theodor Escherich. It lives in the lower intestines of warm blooded animals that include birds and mammals. Its family's scientific name, "enteric", refers to the intestine. Its presence in groundwater is a common indicator of fecal contamination. It is one of the commonly used model organism for bacteria. One

human being passes in average between 100 billion and 10 trillion of individual *E. coli* bacteria in the feces [6].

Proteus vulgaris is a rod-shaped gram negative bacterium (a chemoheterotroph) discovered and isolated by discovered by Gustav Hauser. It lives the intestinal tracts of animals. It is ferments sugar in anaerobic conditions and has the ability to use a wide range of organic molecules in aerobic conditions, refereed as a facultative anaerobe. It can be pathogenic. In humans, it can cause urinary tract infections and wound infections and are found in putrefying materials and in abscesses. It was named after the Greek sea god Proteus as it is pleomorphic and may be present in different sizes and shapes. On the basis of indole production it was differentiated into 3 biogoups. Biogroup one is *P. penneri* (indiole negative), biogroup two and three that are indole positive named together as *P. vulgaris* [7].

MATERIALS AND METHODS

The drugs used in this study, chloroquine and amoxicillin-clavulanic acid combination were procured from the local market (medical shop). Chloroquine was available in the form of a diphosphate salt as a base. The diphosphate is a water soluble, white crystalline, powder bitter in taste. The drugs were powered into dry powder and dissolved in distilled water. The dissolved drugs were then used immediately, they were not stored antimicrobial susceptibility test of these isolates were performed by using Kirby-Bauer’s method. A total of hundred bacterial isolates from clinical samples such as *Escherichia coli* and *Proteus vulgaris* were used in the study. These organisms were available in the Microbiology Department.

A few colonies of the organism to be tested were picked up with a wire loop from the original culture

plate and introduced into a test tube containing 4 ml of tryptose phosphate [8]. These tubes were then incubated for four hours to produce a bacterial suspension of moderate cloudiness. The suspension was then diluted, with distilled water to a density visually equivalent to that of a standard prepared by adding 0.5ml of 1% barium chloride. Petri dishes containing Mueller-Hinton agar were used for anti-microbial sensitivity tests. The plates were dried for about thirty minutes before inoculation. The bacterial broth suspension was streaked evenly in three planes onto the surface of the medium with a cotton swab. Surplus suspension was removed from the swab by rotating it against the side of the tube before the plates were seeded. A stock solution containing 64mg Chloroquine phosphate mL was prepared with distilled water, serial dilution was prepared to obtain 5mL solution, each having different concentration of the drug. Whatman No.1 filter paper was used to prepare discs of 6mm diameter. The discs were then sterilized and each dilution of the drug was added on to the disc at the volume of 10µL per disc, using a micro pipette. Final concentration of Chloroquine phosphate per disc was 64, 53, 42, and 30 µg. Discs will be dried and stored at 4°C. Discs of Amoxicillin/clavulanic acid combination containing 30µg discs were used as control. After the inoculums were dried, the discs were placed on the agar with flamed forceps and gently pressed down to ensure contact. Plates were then incubated immediately. After overnight incubation, the zone diameters were measured on the undersurface with the help of a ruler. The complete inhibition of growth as determined by the naked eye was taken as the end point [9, 10]. The zone diameter were recorded and interpreted accordingly.

RESULTS

Table 1: Zone of inhibition of Escherichia Coli

	Zone of inhibition		Difference between groups			
	Range	Means ± SD	53 µg	42 µg	30 µg	A/C 30 µg
64 µg	8 – 12	10.0 ± 1.1	p<0.01, S	p< 0.01	p< 0.01	p< 0.01
53 µg	6 – 10	8.0 ± 1.1	-	NS	p<0.01	p< 0.01
42 µg	6 – 10	8.0 ± 1.0	-	-	p< 0.01	p< 0.01
30 µg	5 – 9	7.0 ± 0.9	-	-	-	p< 0.01
A/C 30 µg	13 – 15	14.0 ± 0.6	-	-	-	-

ANOVA, F = 198.3 p<0.001, NS ve = 0.98 (5,120)

Newman-Keul’s Range test: LSD = 0.78 p<0.05; LSD = 0.93 p<0.01

In in-vitro tests for chloroquine with *Escherichia coli* [11]: The patterns of inhibition zone are observed at different doses of chloroquine and the control drug combination of amoxicillin-clavulanic acid, and inter group comparisons are done. The range for zone of inhibition is around 8-12 mm in diameter for Chloroquine with strength of 64µg mL⁻¹, with a mean of about 10mm. With strength of 53µg mL⁻¹, the zone of inhibition is between 6 – 10 mm diameters, and with 42µg mL⁻¹ and 30 µg mL⁻¹ of Chloroquine the zone of inhibition is 6 – 10 mm and 5 – 9 mm respectively. There is an increase of inhibition zone along with the

increase of strength of Chloroquine. The control drugs show an inhibition zone of about 13-15mm diameter. When chloroquine strength of 64µg mL⁻¹ was compared with that of 53µg mL⁻¹ and then with 42µg mL⁻¹ and subsequently with 30µg mL⁻¹ of chloroquine a probability value of less than .01 was seen, indicating that the results may be significant. The comparison between 53µg mL⁻¹ and 43µg mL⁻¹ of chloroquine strength has shown no significance. There is a significant difference between all the strengths of chloroquine and the control drug (strength of 30µg mL⁻¹).

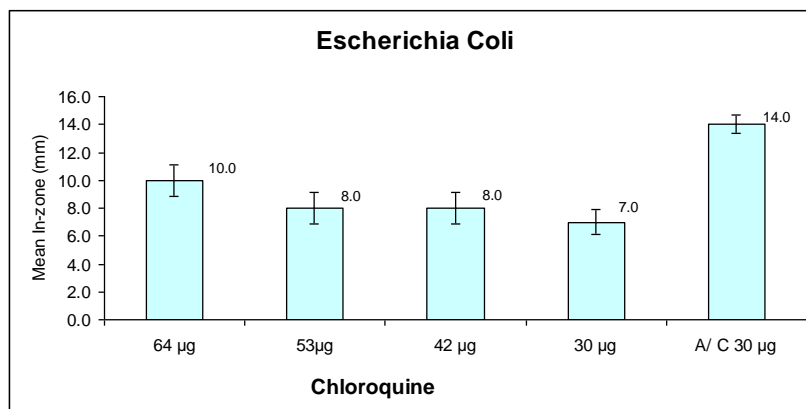


Fig. 1: Mean zone of inhibition of Chloroquine against *E. coli*

The above graph shows the diameter of inhibition on the y-axis, and the strength on the x-axis. In the bar graph there is a small vertical line indicating the standard deviation (SD) the last bar in the graph is that of the control drug combination amoxicillin-clavulanic

acid. The graph shows that the mean inhibition zone by the test drug, chloroquine is less than that of the control drug. The zone of inhibition increases with the increase in the strength of Chloroquine.

Table 2: Zone of inhibition of *Proteus*

	Zone of inhibition		Difference between groups			
	Range	Means ± SD	53 µg	42 µg	30 µg	A/C 30 µg
64 µg	20 – 24	22.0 ± 1.0	P<0.01	P < 0.01	P < 0.01	P < 0.01
53 µg	19 – 21	20.0 ± 0.6	-	NS	P < 0.01	P < 0.01
42 µg	19 – 21	20.0 ± 0.7	-	-	P < 0.01	P < 0.01
30 µg	15 – 17	16.0 ± 0.8	-	-	-	P < 0.01
A/C 30 µg	17 – 19	18.0 ± 0.6	-	-	-	-

ANOVA, F = 205.9 p < 0.001 ve = 0.63
 Newman-Keul's Range test: LSD= 0.62, p < 0.05, LSD = 0.75, p < 0.01

In in-vitro tests of chloroquine with *Proteus vulgaris*: The patterns of inhibition zone are observed at different doses of Chloroquine and the control drug combination of amoxicillin-clavulanic acid, and inter group comparisons are done. The range for zone of inhibition is around 20-24 mm in diameter for chloroquine with strength of 64µg mL⁻¹ with a mean of about 22mm. With strength of 53µg mL⁻¹, the zone of inhibition is between 19-21 mm diameters, and with 42µg mL⁻¹ and 30 µg mL⁻¹ of chloroquine the zone of inhibition is 19-21 mm and 15-17 mm respectively. There is an increase of inhibition zone along with the increase of

strength of Chloroquine. The control drugs show an inhibition zone of about 17-19mm diameter. When Chloroquine with strength of 64µg mL⁻¹ was compared with that of 53µg mL⁻¹ and then with 42µg mL⁻¹ and subsequently with 30µg mL⁻¹ of Chloroquine a probability value of less than .01 was seen, indicating that the results may be significant. The comparison between 53µg mL⁻¹ and 43µg mL⁻¹ of chloroquine strength has shown no significance. There is a significant difference between all the strengths of chloroquine and the control drug (strength of 30µg mL⁻¹).

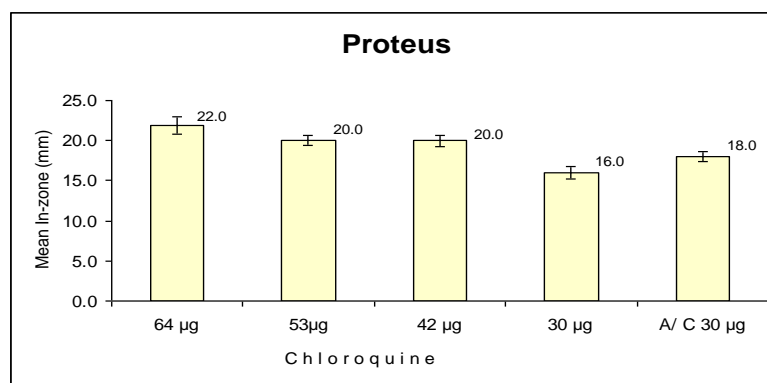


Fig. 2: Mean zone of inhibition of chloroquine against *Proteus*

The above graph shows the diameter of inhibition on the y-axis, and the strength on the X-axis. In the bar graph there is a small vertical line indicating the standard deviation (SD) the last bar in the graph is that of the control drug combination amoxicillin-clavulanic acid. The graph shows that the mean inhibition zone by the test drug, chloroquine is almost similar in diameter to that of the control drug. The zone of inhibition increases with the increase in the strength of chloroquine.

DISCUSSION

Without a standardized reliable method it is very difficult to elucidate a true picture of the bioactivity, therapeutic potential and clinical utility of certain antibacterial drugs. There are several common methods described in the literature to measure bactericidal and bacteriostatic activity of chemotherapeutic agents [12]. In this in vitro study, we have compared the efficacy of chloroquine in different strengths with a standard drug combination of amoxicillin-clavulanic acid which is used frequently in clinical practice.

Advantages of in vitro tests [13]

- Controlled testing conditions
- Lack of systemic effects
- Reduction of variability between experiments
- Testing is fast (and cheap)
- Small amount of test material is required
- Limited amount of toxic waste is produced
- Human cells and tissues can be used
- Transgenic cells carrying human genes can be used
- Reduction of testing in animals

Limitations of in vitro tests [14, 15]

- General toxic effects cannot be assessed (e.g. weight reduction)
- In vivo dose-responses cannot be obtained (for human risk assessment)
- Systemic effects cannot be evaluated
- Interactions between tissues and organs cannot be tested
- Pharmacokinetics cannot be evaluated
- Specific organ sensitivity cannot be assessed
- Chronic effects cannot be tested

Amongst the organisms used to study the antimicrobial efficacy chloroquine, it has been seen that the gram negative bacteria *Escherichia coli* has the least inhibition zone, showing that chloroquine has less efficacy towards it. At the strength of $30\mu\text{g mL}^{-1}$ of chloroquine, the zone of inhibition is around 5 to 9 mm in diameter. As the strength of chloroquine is increased, the zone of inhibition also increases though the initial

rise is marginal; the inhibition zone at the strength of $64\mu\text{g mL}^{-1}$ is around 8 to 12 mm. This indicates that chloroquine is efficacious but when compared with the standard drug combination of amoxicillin-clavulanic acid (A/C), it is far less. The A/C combination shows an inhibition zone of around 13 to 15 mm diameter with strength of $30\mu\text{g mL}^{-1}$. The rod-shaped gram negative bacterium; *Proteus* shows a smaller zone of inhibition than that of the standard drug combination at similar strengths of $30\mu\text{g mL}^{-1}$. The range for zone of inhibition is around 20-24 mm in diameter for chloroquine with strength of $64\mu\text{g mL}^{-1}$. With strength of $53\mu\text{g mL}^{-1}$, the zone of inhibition is between 19-21 mm diameters; and with $42\mu\text{g mL}^{-1}$ and $30\mu\text{g mL}^{-1}$ of chloroquine the zone of inhibition is 19-21 mm and 15-17 mm respectively. There is an increase of inhibition zone along with the increase of strength of chloroquine. The standard drug combination shows an inhibition zone of about 17-19mm diameter.

CONCLUSION

Chloroquine, a 4-aminoquinoline derivative is frequently used as an anti-malarial compound it has also been used in the treatment of Acanthamoeba, Clonorchis sinensis, tenia, fungal, bacterial infection and rheumatoid arthritis. It has also been used as an immunomodulator. On assessment of the antibacterial activity of chloroquine on certain pathogenic bacteria by using disc diffusion technique chloroquine phosphate was found to show a broad range antibacterial activity. It was found effective against *Escherichia coli* which have shown least inhibition zone, *Proteus* is also sensitive to chloroquine.

This was a preliminary in-vitro study, which will require further investigations. However at this stage we could draw a conclusion by saying that this drug can be used as an antimicrobial as it does show efficacy, it is relatively safe and most importantly it is available at a very affordable cost. Further studies need to be taken to determine its antimicrobial dosages in animals and humans.

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