CHAPTER III

ANTIMICROBIAL STUDY OF N[7-CHLORO-4-QUINOLYL] SULFONAMIDES

3.1 INTRODUCTION

Antimicrobial studies of 7-chloro-4-(4'-sulfonamidophenyl amino)-quinolines.

Various workers[1,2] have prepared N-substituted-4-amino-7-chlorohaloquinolines. Many of them possess considerable antimalarial activity. Many amines show remarkable antiplasmodial action. When aromatic heterocyclic nucleus is attached to amino group, the antiplasmodial action increased.[3] Many heterocyclic sulfonamides[4] have been reported which exhibit an excellent antibacterial activity towards S. aureus and E. coli. In substituted sulfonamides, sulfonamide group is responsible for drug activity.[5]

When quinoline nucleus is attached to sulfonamido group, quinoline sulfonamides are formed and certain quinoline sulfonamides have been reported as pharmaceuticals.[5,6,7]

Quinoline compounds, containing at least in 4-position an amino group and in 7-position halogen atom or methyl group, are effective against blood parasites such as those of malaria.[8] Qunoxaline compounds showing antibacterial activity also have been reported.[9,10] Quinaxaline compounds have been used in the treatment of tuberculosis.[11] Many quinoline derivatives possess, number of therapeutic activities, e.g. antiseptic[12], analgesic,[13] typhocidial,[14] germicidal,[15] antitubercular,[16] anthelimintics and antiserotins.[17]

Thus both quinoline derivatives and sulfonamides have therapeutic activities. Hence, when quinoline moiety is attached to sulfonilamide, the derivatives formed must show increased antibacterial activities.

3.2 PRESENT INVESTIGATION :

The present derivatives, in which, 7-chloro-4-quinolyl moiety attached to different substituted sulfonamides report the antimicrobial studies against some representative micro-organism, as these derivatives contain both quinoline and sulfonamido groups.

3.3 Experimental :

All the reagents and solvents used were of Analar or equivalent grade.

3.3.1 Preparation of reagents and stock solutions :

7-chloro-4-quinolyl-sulfonamide derivatives were prepared by dissolving 5 mg in 1 ml. of redistilled ethyl alcohol. Filter paper discs were soaked in these solutions. It was observed that 40 filter paper discs could be soaked in 1 ml. of solution.

3.3.2 Micro organism :

The micro organism used for present studies were pure cultures obtained from the Department of Microbiology, Willingdon College, Sangli.

The following strains were selected for anti-microbial investigation :

(a) Gram positive bacteria

1) Staphylococcus aureus

2) Bacillus subtillis

(b) Gram negative bacteria

1) Escheriachia coli

(c) Fungus strain

1) Aspergillus niger

Non sporulating

sporulating.

3.3.3 Assay Method :

The "disc assay" method used in the present study was same described by Kulkarni P.L. et al.[19] The detail procedure is given below. Nutrient agar was used as a test medium which was prepared by dissolving 'difco agar agar' powder (2.5 gm), peptone (1.0 gm); sodium chloride (0.5 gm) in distilled water. The solution (pH - 7.2 to 7.4) was sterilized by steam in autoclave at 15 lb pressure at 120^{o} for 30 minutes and then poured in sterilized petridish.

For fungal strain Sabouraud's agar containing clucose (4 gm), peptones (1 gm), agar agar powder (2.5 gm) and pH

of solution (5.4).

After cooling (at about 45°C) the medium was poured in sterile petridish. The plates were allowed to solidify. On solid cool surface of agar one drop of lest culture poured at the centre and was spread aspetically using sterile glass spreac

Small and large paper disc were soaked in the solution of compound dried for a while and placed on a surface at equidistance. Separate plates were used for small and large disc. The plates were incubated at 37°C for 24 hours in incubator. The zone of inhibition were measured in millimeters. For mold culture the incubation at room temperature was carried out for 48 hours. All the experiments were carried out in duplicate and average values of diameter of zone of inhibition were recorded. All the operations were carried out in complete aseptic condition to prevent atmospheric contamination. After completion some photographs were taken.

3.4 RESULTS AND DISCUSSION

The results of our present investigation are summarised in Table No. 1 and 1(a). Some typical photographs showing the inhibition zones are presented in plates on pages. Our conclusion based on these results are given below.

1) 7-chloro-4-(4'-sulfonamido-phenyl-imino)quinoline

At 5 mg/ml concentration using small and large disc. S.aureus was inhibited. It has also antimicrobial activity towards E.coli.

It has no antimicrobial activity towards "Bacillus subtilis" using small and large discs.

(2) 7 Chloro-4-(4'-Amino-3'-Methyl, phenyl sulfonimido) quinoline (II).

At 5 mg/ml concentration it has no antimicrobial activity towards B. subtilis. However, it showed activity with only large disc towards E. coli and it also showed with small and large disc activity towards "S. aureus". It has no antimicrobial activity towards "Aspergillus niger".

(3) 7 Chloro 4-(4'-Amino phenyl sulfonimido)-quinoline(III):

At 5 mg/ml concentration it has no antimicrobial activity towards "Bacillus substilis". However with large and small disc it showed activity towards "E. coli". It also showed antimicrobial activity towards "Staphylococcus aureus" using small disc and large disc. It has no antimicrobial activity towards "A. niger".

(4) 7 Chloro 4-(-4'-Amino-2' Methyl phenyl sulfonimido) quinoline (IV) :

At 5 mg/ml concentration using small and large discs it has antimicrobial activity towards "E. coli". It has no antimicrobial activity towards "Bacillus substilis" and "A. niger" using both small and large and small disc respectively.

(5) 7 Chloro-4-(-4'-Acetamido-phenyl sulfonimido) quinoline :

At 5 mg/ml. concentration using small and large disc: It has high antimicrobial activity towards "E. coli". It has also antimicrobial activity towards "S. aureus". It has no antimicrobial activity towards "Bacillus subtilis" and "A. niger" using small and large disc and small disc respectively.

(6) 7 Chloro 4-(-4'-Acetamido 3' Methyl phenyl sulfonimido) quinoline (VI) :

At 5 mg/ml. concentration using large disc only not showed antimicrobial activity towards "E. coli". Similarly at this concentration, "S. aureus" showed antimicrobial activity using both large and small discs. It has no antimicrobial activity towards "A. niger".

(7) 7 Chloro-4-(-4'-Methyl phenyl sulfonimido) quinoline (VII)

At 5 mg/ml. concentration using Large disc only can show antimicrobial activity towards "E.coli" similarly at this concentration "S. aureus" showed antimicrobial activity using both large and small disc. It has no antimicrobial activity towards "A. niger".

Conclusions

From above, we conclude that N-7-chloro-4-quinolyl sulfonamide derivatives have high antibacterial activity against gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria. They are more active towards gram negative than the gram positive bacteria.

According to Table No.1, compounds no. 2,3 & 5 have high potency and 1, 4, 7 have low potency against gram negative bacterial E. coli. Also it is seen that compounds 2, 3, 5 have high potency and 1, 6, 7 low potency towards gram positive bacterial Staphylococcus aureus. The compound No. 4 have no antimicrobial activity towards "S. aureus". All the above derivatives are inactive against "B. substilis" and "A. niger".

All the above derivatives can be used as drugs against gram positive and gram negative bacteria and more effective against gram negative bacteria.

All the above derivatives are inactive against fungus hence they can not be used as an antifungal compounds.

3.5 Comparison of Antimicrobial activity of N-(4 Phenyl-2-quinolyl) sulfonilamides, N-(4 Methyl-2-quinolyl) sulfonilamides and 7 chloro-4-(4'-sulfonimido-phenyl amino) quinoline.

The N-(4-Phenyl-2 quinolyl) sulfonilamides and N-(4-Methyl-2 quinolyl) sulfonilamides are active towards gram positive bacterial (B. substilis) but 7 chloro-4-(4'-sulfonimido-phenyl)-quinoline is inactive towards B. substilis. but it is very active towards S. aureus.

All the above derivatives of sulfonamides are active towards gram negative bacteria E. coli.

In case of N-(4-Methyl-2 quinolyl)-sulfonamides there is no much difference in antimicrobial activity towards both gram positive and gram negative bacteria.

In case of N-(-4-phenyl-2-quinolyl)-sulfonamides activity does not change when 4-phenyl quinolyl moiety is attached to sulfonamides through -NH and $-SO_2NH_2$ Linkage. In case of N-(4-Methyl-2 quinolyl) sulfonamide activity changes, when a linkage is through-SO_2NH the activity is stronger than when through -NH-towards both gram positive and gram negative bacteria.

In case of 7-chloro-4-(-4'-sulfonimido-phenyl-amino-) quinoline, when 7 chloro quinolyl moiety attached to sulfonamide derivative through -NH Linkage the compounds are less active

than when it is attached through $-SO_2NH-$linkage in case of gram +ve and gram negative bacteria. These sulfonamides are inactive towards B. substilis and A. niger.

It is thought that these changes in antibacterial activity of these sulfonamides may be due to electronic and spatial effects of methyl and phenyl groups and the electron of chlorine at withdrawing effect 7 position. But overall it seems there is no much difference in microbial activity of these series of sulfonamide derivatives.

However, derivatives of these sulfonamides are not active towards fungal strain.

Table No.1

Antimicrobial potency of 7 chloro-4-(4'-sulfonimido-phenyl-amino-)quinoline

Inhibition zone diameter in mm.

Organism	I	II	III	IV	V	VI	VII	Disc used in diameter mm.
1. Escherichi coli	15	28	30	14	31	-	13	Large
(Gram -ve)	7	-	16	7	9	-	-	Small
2. Bacillus subtilis	-	-	-	-	-	-	-	Large
(Gram +ve)	-	-	-	-	-	-	-	Small
3. Staphylococcus aureus	16	18	20	-	18	-15	15	Large
(+ ve)	9	7	7	-	10	-	-7	Small
4. Aspergillus niger	-	-	-	-	-	-	-	Large
(Fungal strain)	-	-	-	-	-	-	-	Small

Disc : Small - 5mm.

Large - 11 mm.

Table 1 (a)

Antimicrobial potency of 7-chloro-4-(4'-sulfonamido phenylamino)quinoline

Sr. No.	Name of the compounds	S. aureus (gram +ve)	E. coli (gram -ve)
1.	7-chloro-4-(4'-sulfonamido-phenyl imino)quinoline	+	+
2.	7-chloro-4-(4'-Amino-3'-Methyl phenyl sulfonamido)quinoline	+ +	+ + +
3.	7-chloro-4-(4'-Amino phenyl sulfonamido)quinoline	+ +	+ + +
4.	7-chloro-4-(4'-Amino-2'-methyl phenyl sulfonamido)quinoline	-	+
5.	7-chloro-4-(4'-Acetamido-phenyl sulfonamido)quinoline	+ +	+ + +
6.	7-chloro-4-(4'-Acetamido-3'-Methyl phenyl sulfonamido)quinoline	+	-
7.	7-chloro-4-(4-Methyl phenyl sulfonamido)quinoline	+	+

\+ Low potency (Below 16 mm inhibiting zone)

\+ + Moderate potency (18 - 20 mm inhibiting zone)

\+ + + High potency (28 - 31 mm inhibiting zone)

Table No.2

Antimicrobial Potency of N- (4-Phenyl-2 quinolyl) sulfonimides[21]

Sr.No.	Name of compounds	Bacillus subtilis (gram +ve)	E. coli (gram -ve)
I	2-(4'-Amino Phenyl sulfonimido)-4 phenyl) quinoline	+	+++
II	2-(4'-Amino 3' Methyl phenyl sulfonimido) 4 phenyl) quinoline	+	+++
III	2-(4'-Acetomido phenyl sulfonimide)-4 phenyl) quinoline	+	++
IV	2-(4'-Acetomido 3' Methyl phenyl sulfonimido) 4 phenyl) quinoline	+	+++
V	2-(4'-Methyl phenyl sulfonimido) -4 phenyl) quinoline	++	+++
VI	2-(4' Sulfonamido-phenyl imino)-4-phenyl) quinoline	+	++
VII	2-(-4' sulfonamido, 2' Methyl phenyl imino)-4 phenyl) quinoline	++	+++
VIII	2-(4' sulfonamido, 3' Methyl, phenyl-imino)-4-phenyl)quinoline	+++	+++

+ Low potency (Below 13 mm. inhibiting zone)

++ Moderate potency (14-15 mm inhibiting zone)

+++ High potency (16-18 mm inhibiting zone)

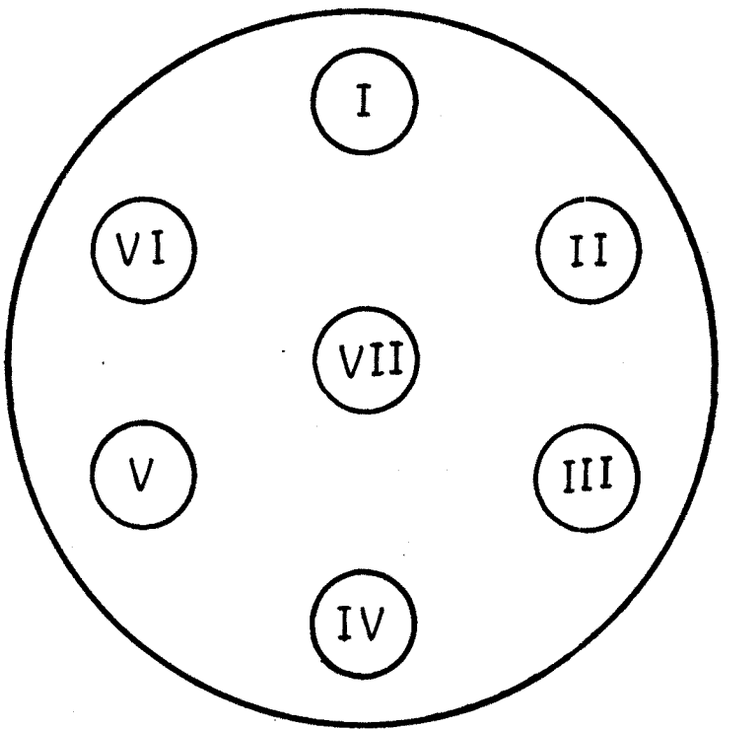
Table No.3

Antibacterial secreening results of N-(-4-Methyl-2 quinolyl) sulfonimides[20]

Sr.No.	Name of compound	Bacillus subtilis	E.coli
1	2-(4'-Aminophenyl sulfonamido -4 Methyl) quinoline	+++	+++
2	2-(-4'-Amino 3' Methyl phenyl sulfonimido 4 Methyl)quinoline	++	++
3	2-(-4'-Amino 2' Methyl phenyl sulfonimido 4 Methyl0quinoline	++	++
4	2-(-4'-Acetamido phenyl sulfonimido -4-Methyl) quinoline	+++	+++
5	2-(-4'-Acetamido, 3' Methyl phenyl sulfonimido -4-Methyl)quinoline	+++	++
6	2-(4'-Acetamido-2'-Methyl phenyl sulfonimido -4-Methyl)quinoline	++	++
7	2-(4' Methyl phenyl sulfonimido 4 Methyl) quinoline	++	+++
8	2-(4' Sulfonamido phenyl imino -4 Methyl) quinoline	++	+++
9	2- (4' sulfonamido-3' Methyl phenyl imino -4 Methyl) quinoline	++	++
10	2-(-4'-Sulfonamido-2'-Methylphenyl imino 4 Methyl) quinoline	++	++

\+ Low potency (25-28 mm. inhibiting zone)
++ Moderate potency (29 mm. inhibiting zone)
+++ High potency (32-35 mm. inhibiting zone)

FOLLOWING FIGURE INDICATES THE POSITION OF COMPOUND IN PHOTOGRAPHS.



I - 7 Chloro-4-[4′-Sulfonimido phenyl imino] quinoline.

II - 7 Chloro-4-[4′-amino 3 Methyl phenyl sulfonimido] quinoline.

III - 7 Chloro-4-[4′-Amino phenyl sulfonimido] quinoline.

IV - 7 Chloro-4-[4′-Amino-2′-Methyl phenyl sulfonimido] quinoline.

V - 7 Chloro-4-[4′-Acetamido phenyl sulfonimido] quinoline.

VI - 7 Chloro-4-[4′-Acetamido-3′-Methyl phenyl sulfonimido] quinoline.

VII - 7 Chloro-4-[4′-Methyl phenyl sulfonimido] quinoline.

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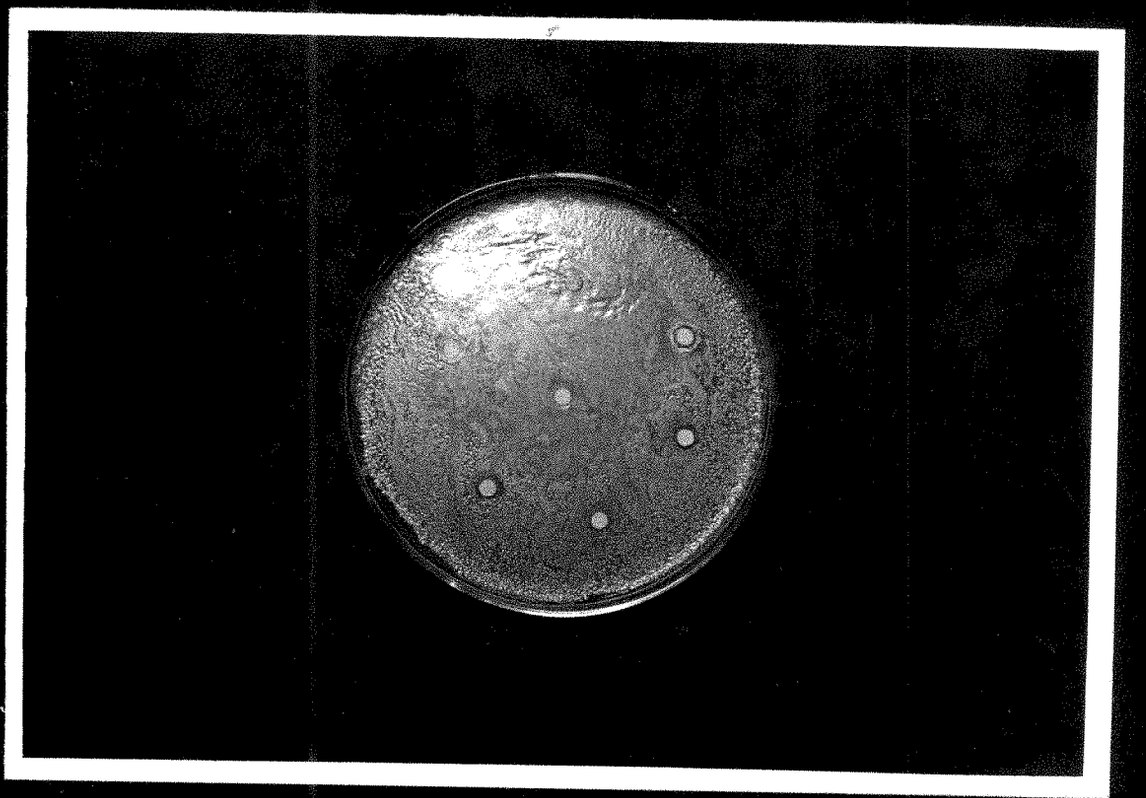
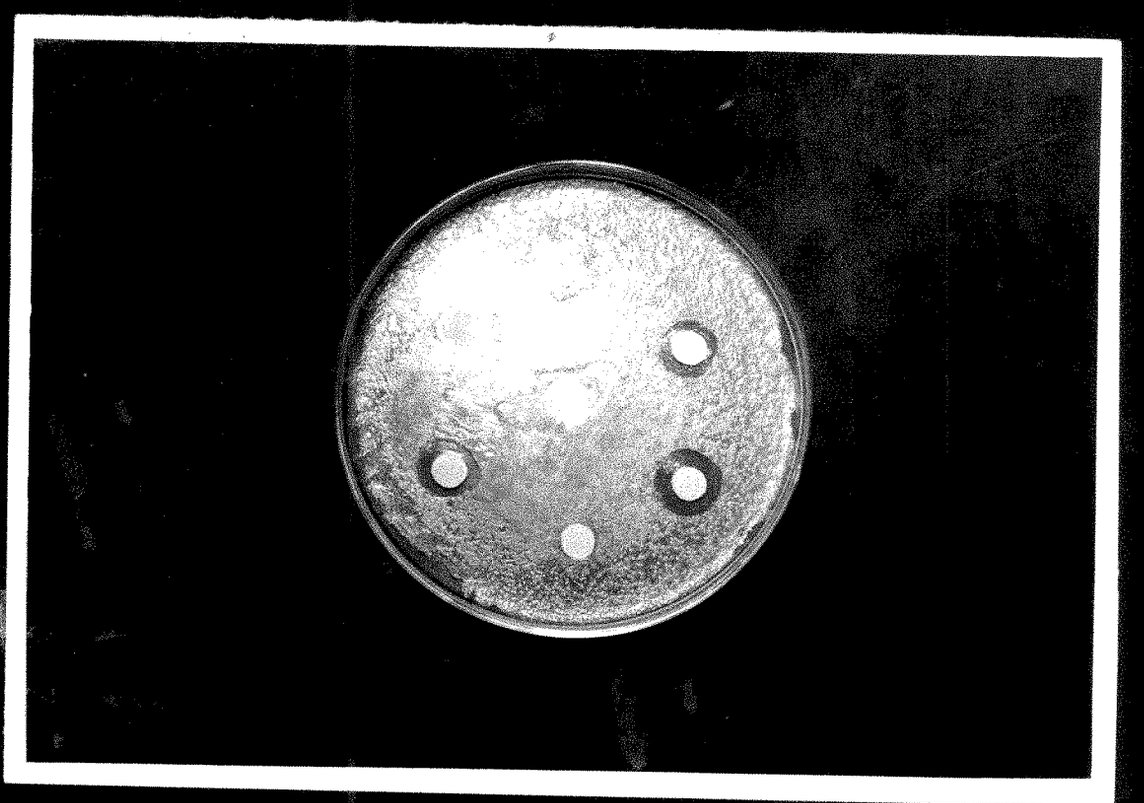
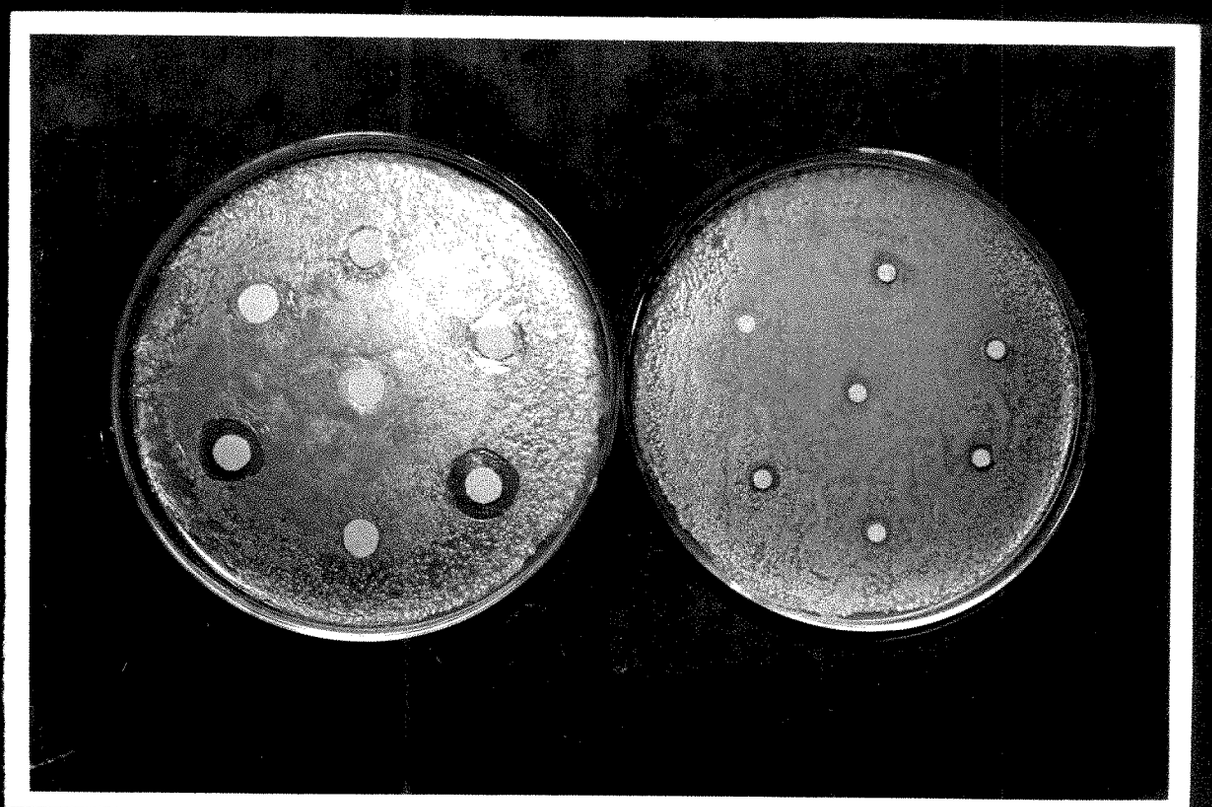


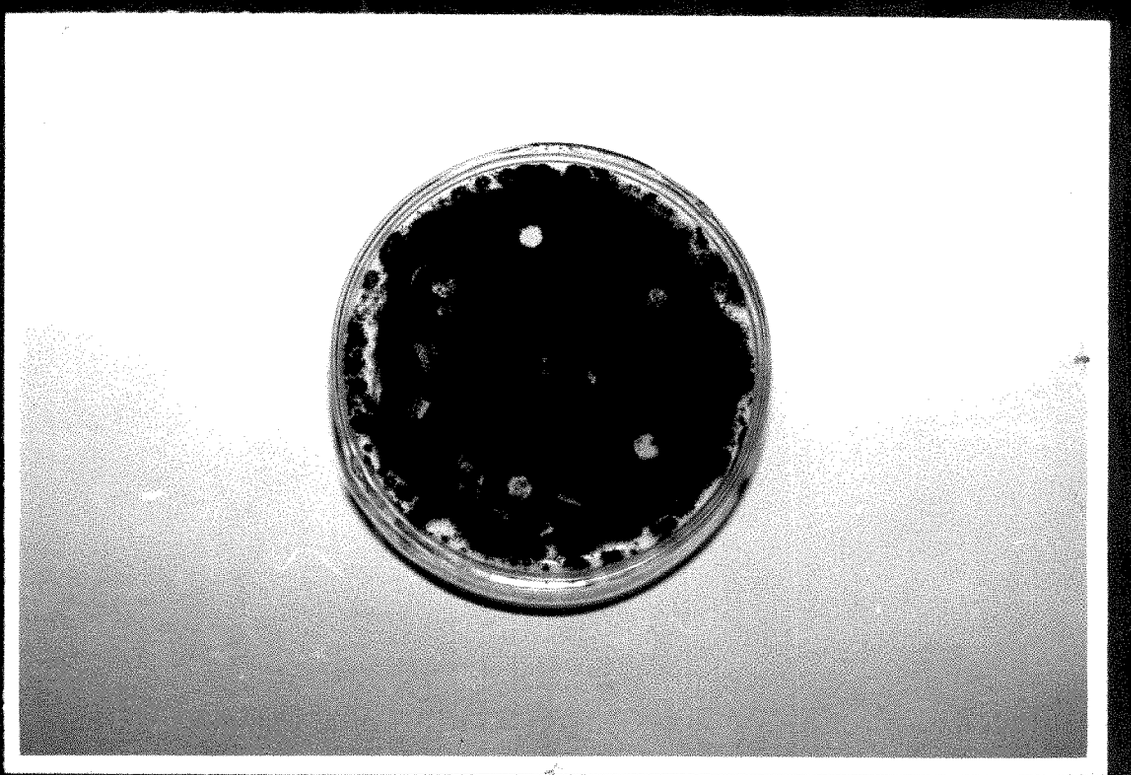
Staphylococcus aureus withsmall dise



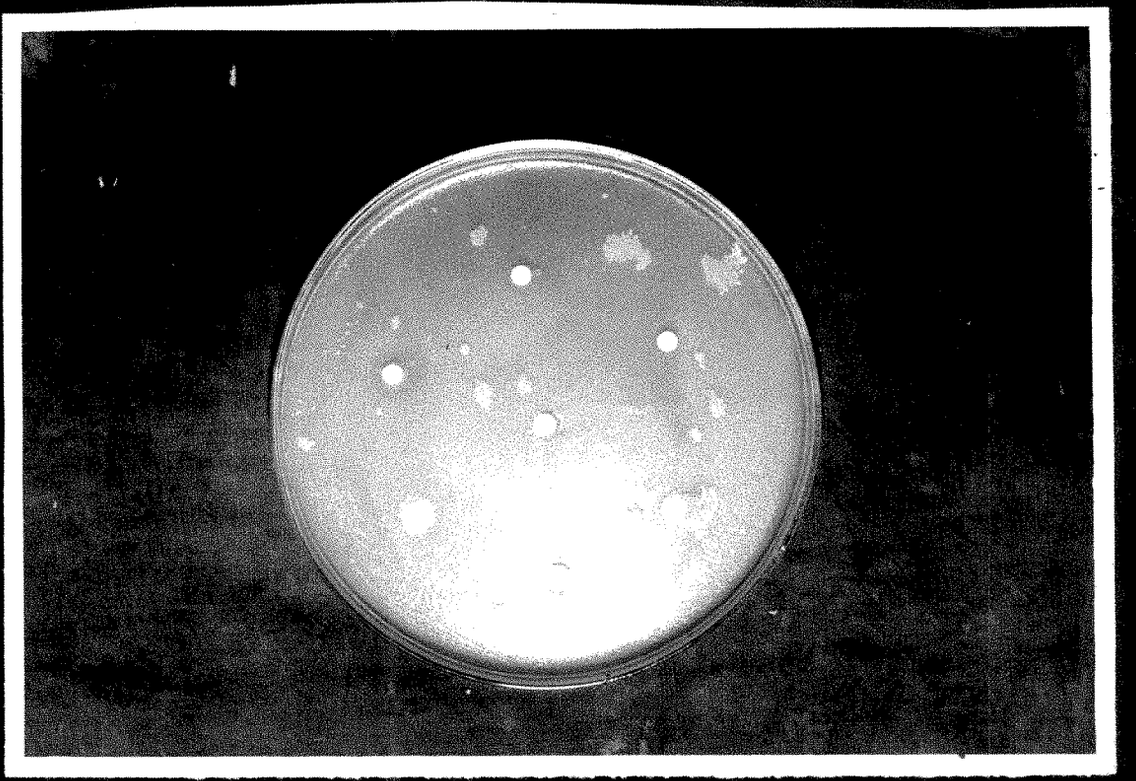
Staphylococcus aureus with large dise



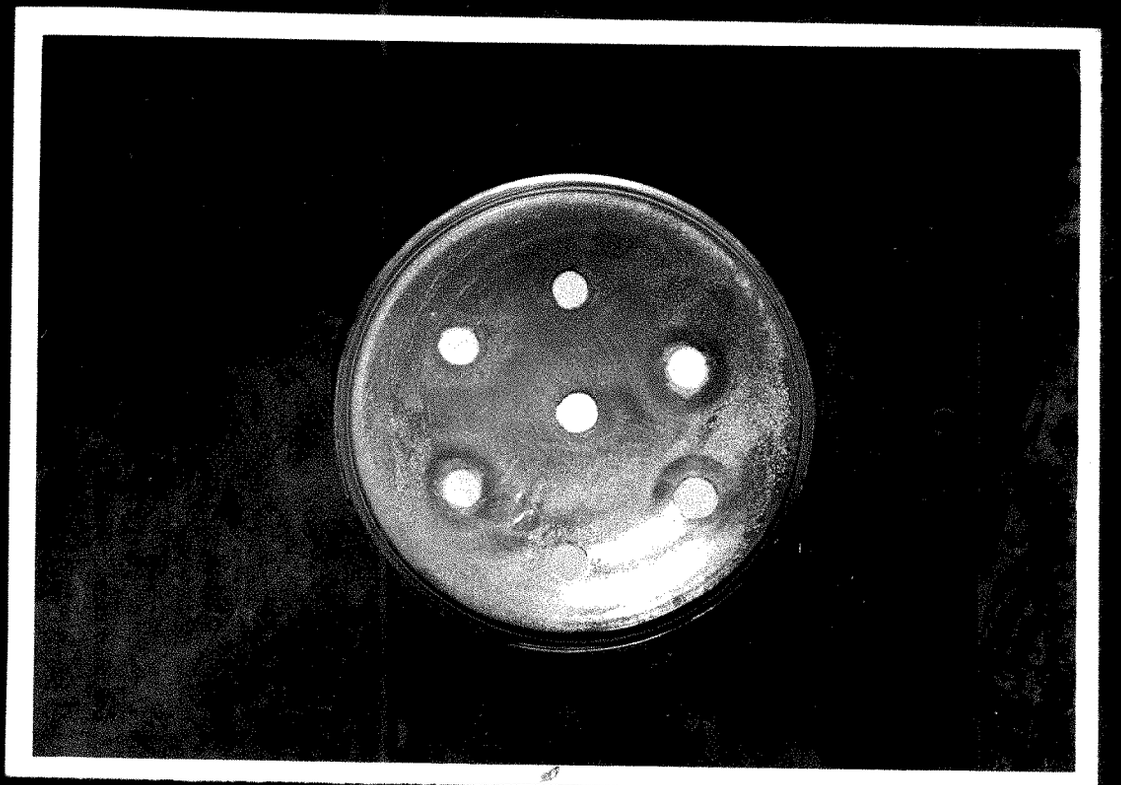
Staphlococcus aureus with small and large disc



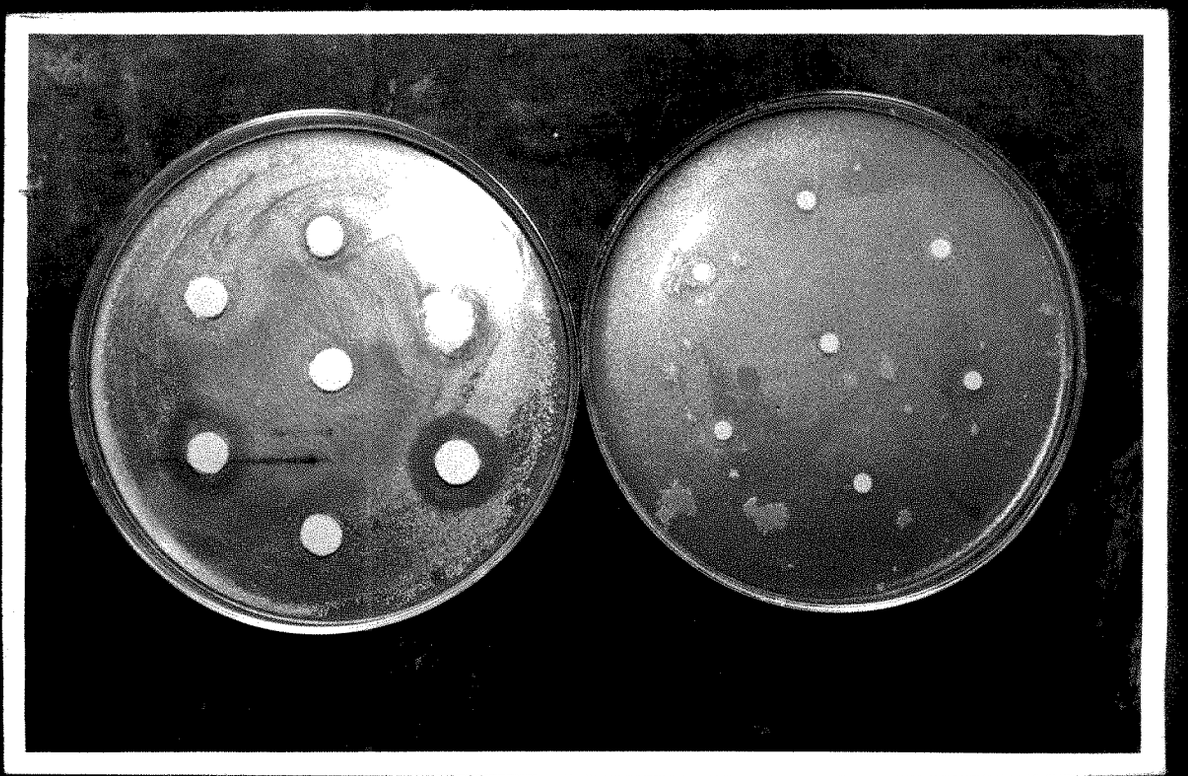
Asperigillus niger with small disc



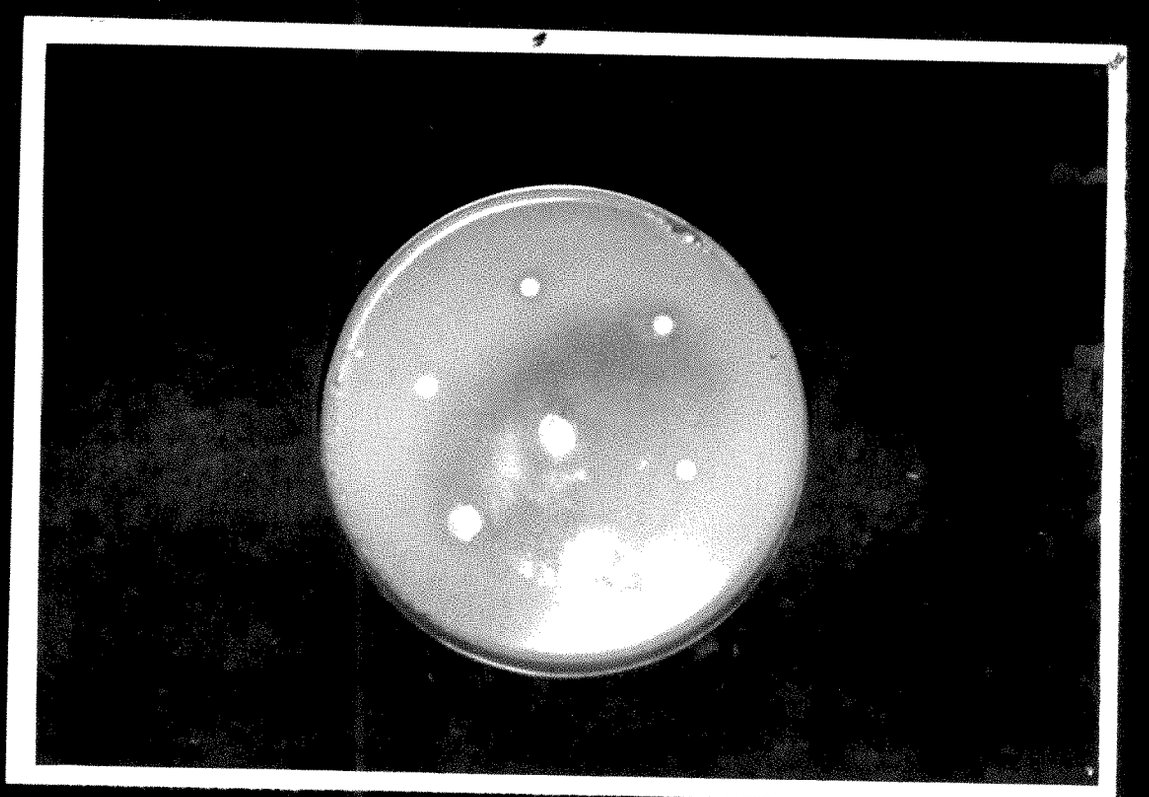
Escheriachia coli with small disc



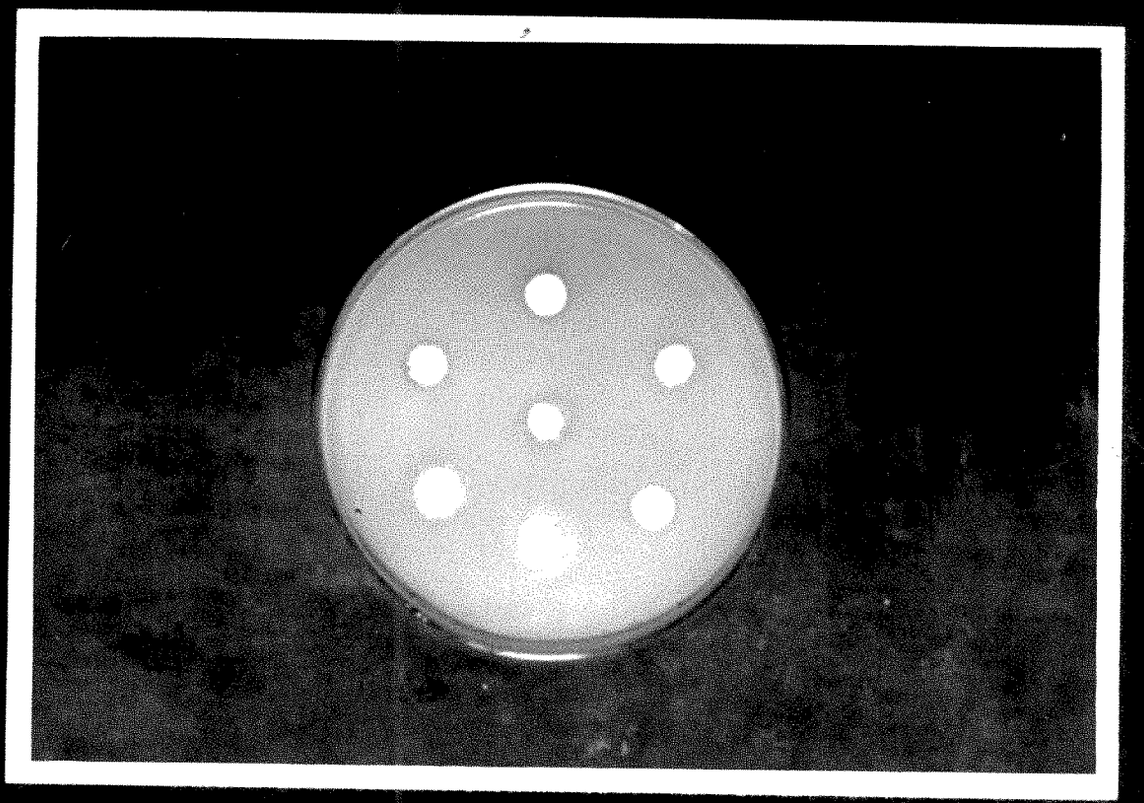
Escheriachia coli with large disc



Escheriachia coli with small and large disc



Bacillus Substilis with small disc



Bacillus Substilis with large disc