



## Optimization of Culture Conditions of MDR Pathogens Isolated From Waste Water

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### ABSTRACT

The optimization of MDR cultures were performed with reference to suitable media, pH, concentration and metal ions. The MDR cultures were isolated from waste water. During the study, out of 16 cultures, 3 bacterial isolates were used for optimization as well as for antibiotic sensitivity tests. The 6 antibiotics were used *Amoxicillin*, *Ampicillin*, *Tetracycline*, *Ofloxacin*, *Chloramphenicol*, *Ciprofloxacin* at lower to higher conc. (10µg- 10mg). The optimization results showed that beef extract media was suitable for the growth of W1 culture, compare to peptone, L.B. and N.B. The suitable temperature was obtained 37 °C compare to 0°C, 4°C and 50°C. The suitable pH was obtained 6 compare to pH 5, 7 and 8 and also the effect of metal ions (1%) were observed on all the cultures and the result obtained maximum for culture S2 in the presence of Ca<sup>++</sup>, compare to Mg<sup>++</sup>, Fe<sup>++</sup>, Pb<sup>++</sup> and Zn<sup>++</sup>.

**Key words:** Optimization, MDR cultures, Antibiotic sensitivity test, Metal ions.

### INTRODUCTION

Waste water from hospitals is usually referred to as hospital waste and is defined as a special category of waste which comprises of all waste, biological or non biological that is discarded from hospitals/health care centers and not intended for further use [1]. Hospital waste consists of both organic and inorganic substances including pathogenic microorganisms. The amount of waste water discharged from hospital varies from hospital to hospital but it has been estimated at 400 to 120 liters/bed/day, [2] reported an estimate on per capita production of waste water in hospital to be 1000 liters/person/day. The important consumption of water in hospitals gives significant volumes of wastewater loaded with microorganisms, heavy metals, toxic chemicals and radioactive elements, the majority of which are pathogenic. About 85% of hospital waste is said to be non hazardous, 10% infective/hazardous and 5% not infective in the United States of America [3]. Meanwhile about 15% of hospital waste is regarded infective in most developed countries. In India, it was reported that the value could increase from 15% to 35% depending on the total amount of hospital waste generated. In Pakistan, about 20% of hospital waste could be found potentially infective or hazardous [4,5]. Hazardous medical waste consists primarily of chemicals and discarded cytotoxic drugs which find their way into the environment due to improper usage and indiscriminate disposal. Their presence in the environment have been reported to pose serious environmental health risk due to their toxic, genotoxic and/or carcinogenic effect [6,7] and could have potential negative effects on the biological balance of natural environment. The present study is carried out to optimize the multi-drug resistance (MDR) pathogens isolated from the different places of Lucknow (waste water). The optimization was done for suitable media, pH, concentration and temperature.

### MATERIALS AND METHODS:

#### Collection of sample:

The water samples were collected from different hospital region as well as wastage region like laundry water of Lucknow. These water samples were designated as sample D2, S2 and W1. Sample D2 = Sample from Devine

hospital, Sample S2 = Sample from Sahara hospital, Sample W1 = Sample from dump water. These samples were isolated for bacteriological analysis by serial dilution and then agar plate culture techniques.

#### Serial dilution:

This method is based on the principle that when soil sample or water sample along with bacterial colonies taken, the result obtained in the form of reduce number of bacterial colonies in order to get pure colonies. The microbes are having importance in the industries for enzyme and antibiotic production.

#### Antibiotics sensitivity test:

It is the method to check sensitivity of antibiotics, if cultures will be resistant for antibiotics then show the growth and if antibiotic is sensitive then they will inhibit the growth of cultures and result can be seen in the form of zone of inhibition. This method was based on agar well diffusion method [8]. Prepare N.A. plates and spread 50µl of isolated culture. Prepare wells and load antibiotics of lower to higher conc. then incubate at 37° C for over night and observed results.

#### Parameters of optimization:

Suitable condition for growth of cultures-

#### Media optimization for bacterial culture growth

The optimization represents the suitable condition for the growth of cultures. The media used were nutrient broth, beef extract, peptone and luria broth.

Prepare media and after autoclaving, inoculate bacterial culture and incubate at 37°C for overnight in shaker incubator and next day take OD at 600 nm.

#### Effect of time:

Prepare nutrient agar and after autoclaving, streak the bacterial cultures and incubate at 37°C for different time incubation-24 hrs, 48 hrs and 72 hrs.

#### Effect of temperature:

Prepare media and after autoclaving, inoculate bacterial culture and incubate at room temperature, 0°C, 4°C, 37°C and 50°C for overnight and next day take OD at 600 nm.

#### Effect of pH:

Prepare media and adjust the pH 5, 6, 7 and 8; after autoclaving, inoculate

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bacterial culture and incubate at 37°C for overnight in shaker incubator and next day take OD at 600 nm.

**Effect of metal ions:**

Prepare media along with 1% metal ions. The metal used were Mg, Fe, Ca, Pb, Zn and after autoclaving, inoculates bacterial culture and incubate at 37°C for overnight in shaker incubator and next day take OD at 600 nm.

**RESULTS:**

**Serial dilution method:**

The serial dilution method was performed in order to get pure and reduce number of bacterial colonies and there were 3 cultures used for further work.



Figure 1: Bacterial colonies in a mixed culture form

**Antibiotics sensitivity test:**

If the cultures were showing growth in the presence of antibiotics then it indicated that cultures were resistant for that antibiotic and if antibiotic will inhibit the growth of that culture in the form of zone of inhibition then it means antibiotic was sensitive.

Table 1: MDR test for D2

Concentration	Amoxicillin	Ofloxacin	Ampicillin	Tetracycline	Ciprofloxacin	Chloramphenicol
10µg	R	R	R	R	R	R
20µg	S	R	R	S	S	R
50µg	R	R	R	R	R	R
100µg	S	R	R	S	S	R
1mg	S	R	S	S	S	R
10mg	S	R	S	S	S	S

R= Resistance, S= Sensitive

Table 1 showed that the 6 antibiotics were used Amoxicillin, Ofloxacin, Ampicillin, Tetracycline Ciprofloxacin and Chloramphenicol and after getting result it was clear that the culture D2 was showing resistant at higher concentration of Ofloxacin and Chloramphenicol.

Table 2: MDR test for S2

Concentration	Amoxicillin	Ofloxacin	Ampicillin	Tetracycline	Ciprofloxacin	Chloramphenicol
10µg	R	R	R	R	R	R
20µg	R	R	R	R	R	R
50µg	R	R	R	S	S	R
100µg	R	R	R	S	S	R
1mg	S	S	S	R	S	R
10mg	S	S	S	R	S	S

R= Resistance, S= Sensitive

Table 2 showed that the 6 antibiotics were used Amoxicillin, Ofloxacin, Ampicillin, Tetracycline Ciprofloxacin and Chloramphenicol and after getting result it was clear that the culture S2 was showing resistant at higher concentration of Tetracycline and Chloramphenicol.

Table 3: MDR test for W1

Concentration	Amoxicillin	Ofloxacin	Ampicillin	Tetracycline	Ciprofloxacin	Chloramphenicol
10µg	R	R	R	R	R	R
20µg	R	R	R	R	R	R
50µg	R	R	R	S	R	R
100µg	R	R	R	S	R	R
1mg	S	S	S	S	R	S
10mg	S	S	S	S	S	S

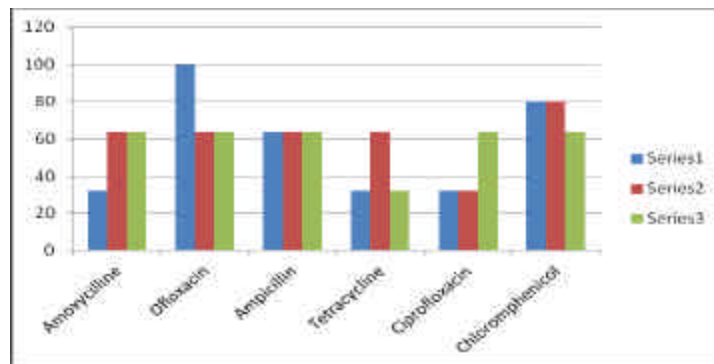
R= Resistance, S= Sensitive

Table 3 showed that the 6 antibiotics were used Amoxicillin, Ofloxacin, Ampicillin, Tetracycline Ciprofloxacin and Chloramphenicol and after getting result it was clear that the culture W1 was showing resistant at higher concentration of Ciprofloxacin.

Table 4: % Activity of resistance for all cultures against antibiotics

Antibiotics	% Activity of culture D2	% Activity of culture S2	% Activity of culture W1
Amoxicillin	32	64	64
Ofloxacin	100	64	64
Ampicillin	64	64	64
Tetracycline	32	64	32
Ciprofloxacin	32	32	64
Chloramphenicol	80	80	64

Table 4 showed that for all antibiotics, all the cultures were showing resistance and against Ofloxacin, it was 100%.



Graph 1: % Activity of resistance of all the cultures against all the antibiotics

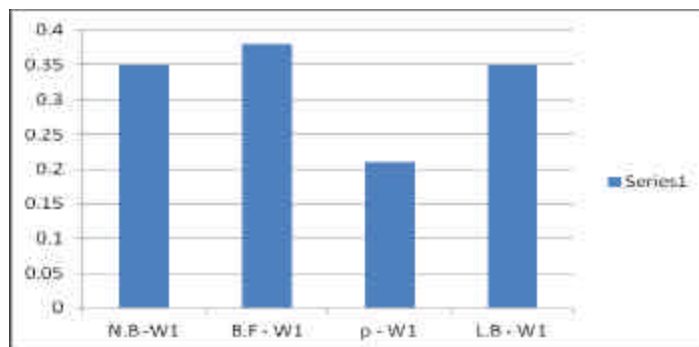
Series1 =D2 culture, series 2= S2 culture and Series 3= W1 culture

Graph 1 showed that the maximum resistance was obtained for culture D2 for Ofloxacin.

**Media optimization:**

Table 5: Media optimization

Culture	N.B	Beef extract	Peptone	L.B
D2	0.05	0.02	0.08	0.05
S2	0.02	0.28	0.17	0.30
W1	0.35	0.38	0.21	0.35



Graph 2: Effect of media on growth culture

Table 5 and graph 2 showed that maximum growth observed for W1 culture in beef extract media.

**Effect of temp:**

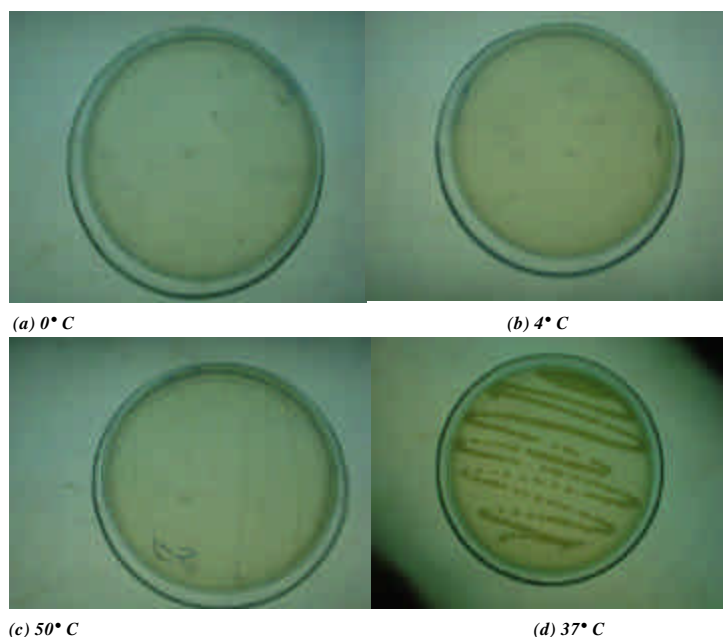
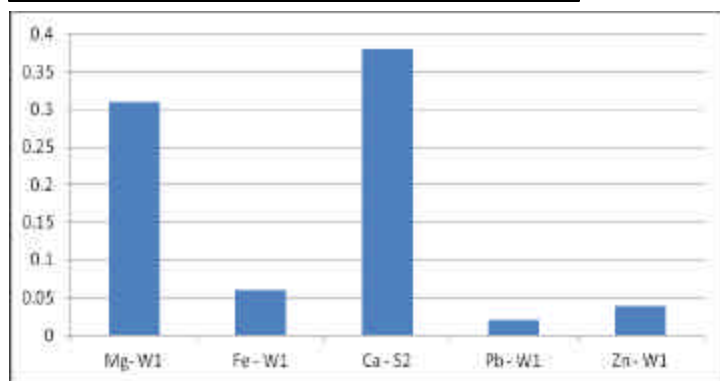


Fig 2 showed that 4 different temp. were used a (0°C), b (4°C), c (50°C), d (37°C), the culture showed the best growth at 37°C.

**Effect of metal ions:**

Table 6: Metal ions were used (1%)

Culture	Mg <sup>++</sup>	Fe <sup>++</sup>	Ca <sup>++</sup>	Pb <sup>++</sup>	Zn <sup>++</sup>
D2	0.15	0.01	0.22	-0.01	0.01
S2	0.12	-0.07	0.38	0.01	-0.03
W1	0.31	-0.06	0.24	0.02	0.04



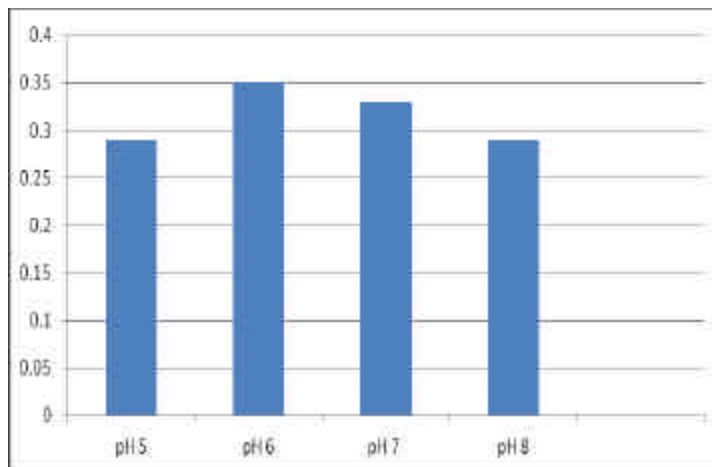
Graph 3: Effect of metal ions

Graph 3 showed the best growth of S2 culture was observed in the presence of Calcium.

**Effect of pH:**

Table 10: pH optimization

Culture	pH-5	pH-6	pH-7	pH-8
D2	0.27	0.26	0.26	0.19
S2	0.18	0.20	0.33	0.29
W1	0.29	0.35	0.29	0.23



Graph 4: Effect of Media growth on pH

Graph 4 showed that the best growth observed in pH 6 in culture W1

**DISCUSSION:**

The MDR cultures are mainly resistant to antibiotics and they showed growth in the presence of antibiotics. Antibiotics are chemotherapeutic agents that have revolutionized the treatment of infectious disease – turning life-threatening diseases into more manageable and treatable conditions. Resistance became a major challenge to the treatment of infectious diseases shortly after the introduction of antibiotics. Bacteria gain resistance through various methods: some bacteria make an antibiotic ineffective before the drug can kill them; some strains alter the drug attack site so that the antibiotic becomes ineffective; some rapidly pump out the antibiotic – antibiotic efflux. Some bacteria have a natural resistance to antibiotics but others become resistant through genetic mutation or by acquiring resistance from another bacterium<sup>[9]</sup>. Optimization of culture conditions included the media composition, temperature, pH, concentration of metal ions used and also time for the growth of cultures. In this present study there were 3 cultures were used from waste water and the name given as W1, S2 and D2. The total 6 antibiotics were used i.e. Amoxicillin, Ampicillin, Tetracycline, Ofloxacin, Chloramphenicol, Ciprofloxacin at lower to higher conc. (10µg-10mg)<sup>[10]</sup>. The best results obtained for Ofloxacin, Ampicillin, Chloramphenicol antibiotics (10µg-1mg). The % resistance activity of all the cultures against all the antibiotics showed that the activity was found 100% for Ofloxacin for D2 culture and 80% for Chloramphenicol for D2 culture<sup>[11,12]</sup>. The optimization results showed that beef extract media was suitable for the growth of W1 culture, compare to peptone, L.B and N.B. The suitable temperature was obtained 37°C compare to 0°C, 4°C and 50°C. The suitable pH was obtained 6 compare to pH 5, 7 and 8 and also the effect of metal ions (1%) were observed on all the cultures and the result obtained maximum for culture S2 in the presence of Ca<sup>++</sup>, compare to Mg<sup>++</sup>, Fe<sup>++</sup>, Pb<sup>++</sup> and Zn<sup>++</sup>.

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