Science Journal of Clinical Medicine
2015; 4(2): 32-40
Published online March 7, 2015 (http://www.sciencepublishinggroup.com/j/sjcm)
doi: 10.11648/j.sjcm.20150402.13
ISSN: 2327-2724 (Print); ISSN: 2327-2732 (Online)

Laboratory dilutions of thioridaxine with potential to enhance antibiotic sensitivity in a multidrug resistant Escherichia coli uropathogen

Otajevwo F. D., Iyabor F. O.

Dept. of Microbiology & Biotechnology, Western Delta University, Oghara, Nigeria

Email address:
fesdotaj@yahoo.com (Otajevwo F. D.), dafotaj@hotmail.com (Otajevwo F. D.)

To cite this article:
Otajevwo F. D., Iyabor F. O.. Laboratory Dilutions of Thioridaxine with Potential to Enhance Antibiotic Sensitivity in a Multidrug Resistant Escherichia Coli Uropathogen. *Science Journal of Clinical Medicine.* Vol. 4, No. 2, 2015, pp. 32-40. doi: 10.11648/j.sjcm.20150402.13

Abstract: This research effort seeks to use doses of thioridaxine to enhance antibiotic sensitivity in a MDR *Escherichia coli* strain. Five axenic (pure) strains of *Escherichia coli* coded EC_1 to EC_5 were obtained from five infected midstream urine samples among several other urine samples inoculated on sterile Cystine Lactose Electrolyte Deficient (CLED) agar with appropriate labeling in the Microbiology and Biotechnology Laboratory of Western Delta University, Oghara, Nigeria and stocked on sterile Nutrient agar slants at 4^{o}C in a refrigerator. Slant cultures were sub-cultured aseptically on fresh sterile CLED agar plates and incubated aerobically at 37^{o}C for 24hrs to confirm *Escherichia coli* strains. Gram staining, indole production, methyl red test, voges praskaeur and citrate utilization tests were done on the resulting colonies to further confirm the strains as *E.coli*. Antibiotic susceptibility test was done by agar disc diffusion method on all confirmed strains on sterile Mueller- Hinton agar plates before and after treatment with laboratory dilutions of thioridaxine. Only *E.coli* strain 2 (EC_2) was multidrug resistant as it resisted 4(44.4%) of the antibiotics used which were cefuroxime, nalidixic acid, augmentin and tetracycline. Other strains resisted 1-2 antibiotics. The highest (15.6±20.6mm) and least (2.0±8.1mm) zones of inhibition by all five strains were recorded for ofloxacin and cefuroxime respectively. Whereas all five uropathogen strains resisted augmentin, they were sensitive to ciprofloxacin, ofloxacin (both being fluoroquinolones), gentamicin, chloramphenicol and nitrofurantoin. After treatment with 2000-2240ug/ml laboratory dilutions of thioridaxine, ≤50.0% loss of resistance was recorded for 2040ug/ml, 2160ug/ml and 2240ug/ml dilutions. Thioridaxine dilution of 2040ug/ml induced 250% and 90% resistance losses of EC_2 to ciprofloxacin and nitrofurantoin respectively with an overall mean±S.E loss of 68.0±24.4%. Resistance losses of 112.5%, 130.0% and 100.0% to ciprofloxacin, nitrofurantoin and chloramphenicol respectively were recorded after 2160ug/ml treatment and 68.5±16.3% overall loss of resistance. Thioridaxine dilutions of 2240ug/ml induced 55.6±25.0% overall loss of resistance with a corresponding 50.0%, 50.0%, 58.1%, 70.0% and 50.0% resistance losses with gentamicin, ciprofloxacin, ofloxacin, nitrofurantoin and chloramphenicol respectively. Less than 50% resistance losses were recorded for 2000, 2080, 2120 and 2200ug/ml dilutions. Minimum inhibitory concentration of chloramphenicol was lowered by 2080ug/ml, 2160ug/ml and 2240ug/ml dilutions by two-fold (15ug), two-fold (15ug) and four-fold (7.5ug) respectively. The medical/ chemotherapeutic implications of these findings are discussed.

Keywords: Invitro, Dilutions, Thioridaxine, Enhance, Antibiotic, Sensitivity, MDR *E.coli*

1. Introduction

Antibiotics resistance is not a new phenomenon. However, the current magnitude and speed with which it is developing is a cause for global concern (Namita *et al*., 2012). According to WHO (2012), antimicrobial resistance is on the rise in Europe and all over the world with gradual loss of first line antimicrobials. Epidemiological studies have suggested that antibiotic resistance genes emerge in microbial populations within 5 years of the therapeutic introduction of an antibiotic (Chakrabarty *et al.,* 1990).

Hence, numerous classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance often as a result of the selective pressure of their daily usage (Osakay *et al.,* 2009*)*. This selective pressure can be attributed to indiscriminate use of antibiotics, complex socio- economic behavioural antecedents and dissemination of drug resistant pathogens in

human medicine (Okeke *et al.,* 1999*)*. Moreover, the disappointing lack of new antimicrobial agents has led to overuse of existing ones thus leading to the emergence of multi-resistant pathogens (McGowan, 2006).

Therefore, as the proliferation of multidrug resistant pathogens continue unavoidably within and around us, it is important that their resistance trend be put under check through intensive research and antibiotic surveillance (Akortha and Filgona*,* 2009). The primary causes of antibiotic resistance in bacteria are mobile elements called plasmids and conjugative transposons. Plasmids are extra chromosomal DNA elements that have the capacity to replicate independently of the chromosome of the bacterial cell (Madigan *et al*., 2003). Resistance plasmids or R plasmids code for enzymes that can inactivate antibiotics, prevent the uptake of an antibiotic or pump out the particular antibiotic (Neu, 1989). Other causes of antibiotic resistance are efflux pumps, mutation, under dosage or use of drugs without prescription (Amaral *et al*., 2013). Plasmids carry genes some of which code for beta-lactamase or extended spectrum beta lactamases which can inactivate or degrade drugs thus rendering them ineffective (Amaral *et al*., 2013).

Curing is the process of removing plasmids from a bacterial cell (Trevors, 1986). The resulting bacterial organism then becomes sensitive to the selective agent and it was initially thought that this phenomenon would proffer solution in controlling the development of antibiotic resistance in formerly antibiotic susceptible bacteria. Novobiocin, ethidium bromide, acriflavine, acridine orange, ascorbic acid and elevated temperatures have been used as curing agents (Ramesh *et al*., 2000). Physical treatments, chemical compounds and growth conditions may increase the frequency of elimination of drug resistant R-plasmids, resulting in sensitive cells that were previously resistant to antibiotics (Lakshmi *et al*., 1981). DNA intercalating dyes (ethidium bromide), sodium dodecyl sulphate (SDS), antibiotics, thymine starvation and elevated temperatures have been used as curing agents (Chakrabarrty *et al*., 1984; *Gupta et al*., 1980; *Obaseki-Ebor*, 1984; *Reddy et al*., 1986).

It has been reported that phenothiazines have the ability to control overexpression of efflux pump systems and thus are able to remove or reduce antibiotic resistance (Viveiros *et al*., 2010; *Amaral et al*., 2013). Mukherjee *et al*. (2012) reported the use of 1000-3000ug/ml dilutions of a type of phenothiazine and an anti-psychotic drug called thioridaxine to cure a multidrug resistant strain of *Pseudomonas aeruginosa.*

Multidrug resistance is now common among familiar pathogens such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae*, etc. *Escherichia coli* causes about 85% of community acquired UTIs, 50% of hospital acquired (nosocomial) UTIs and more than 80% cases of uncomplicated pyelonephritis (Bergeron, 1995). The antibiotic sensitivities of different strains of *E.coli* vary widely. As a gram negative organism, *E.coli* is resistant to many antibiotics that are effective against gram positive organisms (Johnson *et al.,* 2006). *E.coli* bacteria often carry multidrug resistance plasmids and under stress, readily transfer those plasmids to other species (Salyers *et al*., 2004).

Extended spectrum beta-lactamase producing *E.coli* are highly resistant to an array of antibiotics and infections by these strains of *E.coli* are highly difficult to treat (Paterson and Bonomo, 2005). Virulent strains of *E.coli* can cause infantile gastro-enteritis, pelvic inflammatory disease and neonatal meningitis (Todar*,* 2007).

Treatments that increase frequency of elimination of plasmids will certainly enhance sensitivity (effectiveness) of antibiotics in situ. There is no published current work on use of laboratory dilutions of thioridaxine in the treatment (curing) of a multidrug resistant *Escherichia coli* uropathogen. The focus of this work therefore, is the use of laboratory dilutions of thioridaxine to enhance the antibiotic sensitivity of a multidrug resistant *Escherichia coli* uropathogen with the following objectives:

1 Determine the antibiograms of five selected *Escherichia coli* pure culture strains obtained from cultures of midstream urine samples after 37^{o}C incubation for 24hrs with the aim of selecting a multi-antibiotic resistant strain.
2 Determine the antibiotic susceptibility profiles of a selected MDR *E.coli* strain in terms of ≤50% resistance loss after treatment with laboratory dilutions of thioridaxine (i.e. 2000-2240ug/ml).
3 Show a summary of data of ≤50% resistance loss by thioridaxine dilutions after treatment on the MDR *E.coli* strain.
4 Determine thioridaxine dilutions' effect(s) on the minimum inhibitory concentration (MIC) of a selected antibiotic that recorded borderline loss of resistance (i.e. between 45-49% as borderline to ≤ 50%).

2. Materials and Methods

2.1. Sampling

Five pure (axenic) isolates (strains) of *Escherichia coli* were obtained from 24hr Cystine lactose Electrolyte Deficient (CLED) agar plate cultures. The agar plate medium used was one of several agar plates which had been inoculated with freshly voided midstream urine samples by a graduating student working on urinary tract infection in the Microbiology and Biotechnology laboratory of Western Delta University, Oghara.

The status of the *E.coli* isolates (strains) was re-confirmed by gram reaction, biochemical and sugar fermentation tests by standard methods (Cowan and Steel*,* 1993). After confirmation, all gram negative, raised, entire, circular, motile, indole positive, methyl red positive, voges praskauer negative, citrate negative, urease negative, lactose and glucose fermenting colonies were stocked (i.e. streaked aseptically) on sterile nutrient agar (LabM, UK) slants and incubated at 37^{o}C for 24hrs. The resulting cultures were kept at 4^{o}C in the refrigerator for further use after appropriate labeling. The five bacterial uropathogens were then subjected

to antibiotic sensitivity testing before treatment with laboratory dilutions of thioridaxine.

2.2. Antibiotic Sensitivity Testing

The five stocked *E.coli* strains were first subcultured on sterile CLED agar medium and incubated at 37^{o}C for 24hours. Antibiotic sensitivity testing was then carried out on the resulting pure culture colonies using the agar disc diffusion method on sterile Mueller-Hinton agar (MHA) plates (Bauer *et al*., 1966). A loopful of each colony of the uropathogens was picked aseptically using a flamed and cooled wire loop and placed in the centre of the sterile MHA plates. This was then spread all over the plates applying the caution of not touching the edges of the plates. The seeded plates were allowed to stand for about 2 minutes to allow the agar surface to dry. A pair of forceps was flamed and cooled and used to pick an antibiotic multidisc (Abitek, Liverpool) containing augmentin (30ug), ofloxacin (5ug), gentamicin (10ug), nalidixic acid (30ug), nitrofurantoin (200ug), tetracycline (25ug), chloramphenicol (30ug), ciprofloxacin (10ug) and cefuroxime (30ug). The discs were placed at least 22.0mm from each other and 14.0mm from the edge of the plates (Ochei and Kolkhatkar, 2008). Antibiotic discs were selected on the basis of their clinical importance and efficacy on various pathogenic strains of *Escherichia coli*. The seeded plates were allowed to stand for 10mins before incubation (Mbata, 2007).

At the end of incubation, the diameters of the zones of inhibition from one edge to the opposite were measured to the nearest millimeter using a transparent ruler (Byron *et al*., 2003). Strains that showed resistance against three antibiotics and above were termed multiple drug resistant strains (Jan *et al*., 2004) and were noted and used further.

2.3. Preparation of Laboratory Dilutions of Thioridaxine

Thioridaxine, a phenothiazine (also known as 2-Methylmercapto-10-(2-N-methyl-2-piperidyl-ethyl phenothiazine) dilutions of 2000-2240ug/ml were chosen based on the lethal Dose-50 (LD_{50}) of 956-1034mg/kg administered orally on rats as reported by Barth *et al*. (2006) and in line with a similar study carried out by Mukherjee *et al*. (2011). Laboratory thioridaxine dilutions of 2000ug/ml, 2040ug/ml, 2080ug/ml, 2120ug/ml, 2160 ug/ml, 2200ug/ml and 2240ug/ml were therefore prepared using RV/O where stock or original concentration of thioridaxine used was 50mg tablet (Southwood Pharmaceuticals, UK). The 50mg tablet was originally dissolved in 10ml sterile water to give 5mg/ml which is equivalent to 5000ug/ml. To obtain 2000ug/ml dilution, 2ml of stock or original drug (5000ug/ml thioridaxine) was mixed or diluted with 3ml sterile water.

To obtain 2040ug/ml dilution, 5.1ml of stock was mixed with 7.4ml of sterile water. A mixture of 5.2ml of stock drug solution with 7.3ml of sterile diluent resulted in a dilution of 2080ug/ml and 2120ug/ml dilution was obtained by mixing 5.3ml of original drug solution with 7.2ml of diluent (sterile water). To obtain 2160ug/ml dilution, 5.4ml stock drug solution was mixed with 7.1ml of diluent, while a mixture of 1.1ml of stock with 1.4ml of diluent gave a dilution of 2200ug/ml. Lastly, 2240ug/ml was obtained by mixing 5.6ml of stock with 6.9ml of diluent.

2.4. Growing Broth Culture of MDR Escherichia coli Strain 2 (EC_2)

The stock culture of EC_2 was selected from among the initial five stocked strains. An inoculum of EC_2 was aseptically picked from its slant stock culture using flamed and cooled wire loop and inoculated into 10ml sterile Nutrient broth (LabM, UK). The inoculated broth was incubated at 37^{0}C for 18hrs. The resulting turbid broth culture was then diluted according to a modified method of Shirtliff *et al.* (2006). Using a sterile pipette, 0.1ml of broth culture was mixed with 19.9ml (1:200 dilution) of sterile Nutrient broth. This was properly mixed and was used as working inoculum and should contain 10^5 to 10^6 organisms and used within 30 minutes (Ochei and Kolhatkar, 2008).

2.5. Treatment of EC_2 Uropathogen with Prepared Thioridaxine Dilutions

The treatment of MDR *Escherichia coli* strain 2 with the prepared thioridaxine dilutions was done according to a modified method by Byron *et al*. (2003). Using a sterile pasteur pipette, 0.5ml aliquot of the diluted overnight broth culture of EC_2 uropathogen was added to 4.5ml sterile molten Nutrient agar (LabM, UK) and mixed. The various prepared dilutions (one at a time) of thioridaxine were then added in 0.5ml volume. The set up for each dilution was then poured on top of sterile hardened or set 2% Nutrient agar plates and left to set. The same antibiotic multidiscs used before treatment were then picked (using flamed and cooled pair of forceps) and impregnated on the set agar overlay plates. Plates were incubated at 37^{0}C for 24hrs. Measurement of diameter of zones of inhibition was taken and recorded (NCCLS, 2000).

2.6. Determination of Effect of Thioridaxine Dilutions Treatment on MIC of Chloramphenicol

Serial doubling dilutions of chloramphenicol (using its MIC of 30ug as a basis) was carried out. Chloramphenicol was chosen for this assay because it showed a mean ± S.E resistance loss of 45.0 ± 20.0 across all the dilutions and 45% is a borderline of ≤50.0% which is the benchmark for the purpose of this study. The idea is that any thioridaxine dilution that can reduce the MIC may enhance its sensitivity and therefore, loss of resistance is likely to shore up from 45%. Sterile cotton wool plugged test tubes numbering 11 (eleven) were set up on a test tube rack and labeled 1-11. Using a sterile pipette, 1ml of sterile nutrient broth was dispensed into tubes 2 to 7. Two milliliters of Nutrient broth was dispensed into tube 8 as Nutrient broth control. Tubes 2-7 were then labeled with chloramphenicol concentrations of 120ug/ml, 60ug/ml, 30ug/ml, 15ug/ml, 7.5ug/ml, 3.75ug/ml and 1.88ug/ml. A 250mg capsule of chloramphenicol was

dissolved in 10ml of sterile water and diluted to 240ug/ml using RV/O in a sterile 200ml transparent bottle. From this 240ug/ml chloramphenicol preparation, 2ml volume was pipetted into tube 1. From tube 1, one milliliter was pipetted into tube 2 and mixed and 1.0ml was pipetted into tube 3 and mixed. From tube 3, 1.0ml was pipetted into tube 4. Finally, 1.0ml was pipetted into tube 7, mixed and 1.0ml was pipetted out and discarded. The diluted *E.coli* inoculum was then dispensed in 0.5ml volume into tubes 2 to 7. Into tube 10, two milliliters of the diluted broth culture was dispensed as inoculum control. Into tube 9, two milliliters of the 240ug/ml chloramphenicol diluted drug was dispensed as drug control. The first dilution of thioridaxine (2000ug/ml) was then added (in 0.5ml volume) to each tube and the content of each tube was properly mixed. The set up was repeated for each of the other dilutions of thioridaxine.

All tubes were incubated in a water bath at 37^{o}C for 24hrs. The MICs as affected by each thioridaxine dilution were read and recorded.

3. Results

Table 1 shows antibiograms of *Escherichia coli* strains 1-5 isolated from mid-stream urine samples of which their sensitivity responses to cefuroxime, nalidixic acid, gentamicin, ciprofloxacin, tetracycline, chloramphenicol, ofloxacin, augmentin and nitrofurantoin are shown. *E.coli* strains 1 and 4 resisted two antibiotics each (cefuroxime and augmentin) while EC_3 and EC_5 each resisted one antibiotic which incidentally was augmentin. Only *E.coli* strain 2 resisted three antibiotics and these were cefuroxime, nalidixic acid and augmentin.

The highest (15.6±20.1mm) and least (2.0±8.1mm) zones of inhibition by all five strains were recorded for ofloxacin and cefuroxime respectively. All the five *E.coli* uropathogens resisted augmentin.

Because *E.coli* strain 2 resisted three antibiotics, it was considered a multidrug resistant uropathogen and was used further in the study.

Table 1. *Sensitivity Profile of five Escherichia coli strains before treatment with laboratory dilutions of thioridaxine after incubation at 37^{o}C for 24hrs.*

Strain Code	Zones of inhibition (mm)								
	CRX	NAL	GEN	CPR	TET	CHL	OFL	AUG	NIT
EC_1	0.0	7.0	15.0	10.0	5.0	6.0	15.0	0.0	9.0
EC_2	0.0	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
EC_3	4.0	5.0	7.0	12.0	4.0	8.0	11.0	0.0	9.0
EC_4	0.0	10.0	13.0	15.0	8.0	10.0	16.0	0.0	12.0
EC_5	6.0	12.0	6.0	6.0	7.0	11.0	12.0	0.0	8.0
Mean±S.E	2.0±8.1	6.8±11.2	10.6±7.2	10.2±8.1	4.8±6.1	8.2±5.6	15.6±20.1	0.0	9.6±11.3

CRX= Cefuroxime, NAL=Nalidixic Acid, GEN=Gentamicin, CPR=Ciprofloxacin, TET= Tetracycline, CHL=Chloramphenicol
OFL=Ofloxacin, AUG=Augmentin, NIT = Nitrofurantoin, EC_1- EC_5= *Escherichia coli* strains 1-5.

Table 2. *Thioridaxine dilutions that induced ≤50.0% loss in resistance after treatment on a multidrug resistant Escherichia coli uropathogen.*

Thioridaxine lab dilutions	Selected Antibiotics (% improvements in sensitivity)							
	Treatments	NAL	GEN	CPR	TET	CHL	OFL	AUG	NIT
2000ug/ml	Before	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
	After	0.0 (0.0)	14.0 (16.7)	10.0 (25.0)	0.0 (0.0)	13.0 (115.0)	25.0 (4.2)	0.0 (0.0)	19.0 (90.0)
2040ug/ml	Before	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
	After	0.0 (0.0)	16.0 (33.3)	28.0 (250.0)	0.0 (0.0)	6.0 (0.0)	28.0 (16.7)	0.0 (0.0)	19.0 (90.0)
2080ug/ml	Before	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
	After	0.0 (0.0)	18.0 (50.0)	16.0 (50.0)	0.0 (0.0)	9.0 (50.0)	27.0 (12.5)	0.0 (0.0)	15.0 (50.0)
2120ug/ml	Before	0.0	12.0	8.0	0.0	0.0	24.0	0.0	10.0
	After	0.0 (0.0)	12.0 (0.0)	14.0 (75.0)	0.0 (0.0)	0.0 (0.0)	36.0 (50.0)	0.0 (0.0)	18.0 (18.0)
2160ug/ml	Before	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
	After	0.0 (0.0)	12.0 (0.0)	17.0 (112.5)	0.0 (0.0)	12.0 (100.0)	27.0 (12.5)	0.0 (0.0)	23.0 (130.0)
2200ug/ml	Before	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
	After	0.0 (0.0)	12.0 (0.0)	11.0 (37.5)	0.0 (0.0)	6.0 (0.0)	26.0 (8.3)	0.0 (0.0)	16.0 (16.0)
2240ug/ml	Before	0.0	12.0	8.0	0.0	6.0	24.0	0.0	10.0
	After	0.0 (0.0)	18.0 (50.0)	16.0 (50.0)	0.0 (0.0)	9.0 (50.0)	38.0 (58.1)	0.0 (0.0)	17.0 (70.0)

Table 2 shows the sensitivity profile of EC_2 (*Escherichia coli* strain 2) after treatment with thioridaxine dilutions. The

uropathogen was resistant to nalidixic acid, cefuroxime, tetracycline and augmentin. The uropathogen EC_2 was sensitive to gentamicin, ciprofloxacin, chloramphenicol, ofloxacin and nitrofurantoin with 12.0mm, 8.0mm, 6.0mm, 24.0mm and 10.0mm zones of inhibition respectively (Table 1). Table 2 also shows data of zones of inhibition of *E.coli* strains 2 after treatment with laboratory dilutions of thioridaxine. Zones of inhibition before and after treatment were also mathematically computed to obtain ≤50.0% loss in resistance (i.e. improvement in sensitivity). Treatment with 2000ug/ml thioridaxine recorded a 115% loss of resistance to chloramphenicol, 90.0% loss in resistance to nitrofurantoin, less than 20% and 30% resistance losses to gentamicin and ciprofloxacin respectively,

Sensitivity improvement or resistance loss as high as 250.0% was recorded for ciprofloxacin after treatment with 2040ug/ml dilution of the chemical agent. Gentamicin was distantly next with a less than 40.0% loss of resistance. After 2080ug/ml thioridaxine treatment, 50% resistance loss was recorded for gentamicin, ciprofloxacin, chloramphenicol and nitrofurantoin each. The same curing treatment resulted in a less than 15% resistance loss to ofloxacin. Seventy five percent (75%), 50% and 80% resistance losses were recorded for ciprofloxacin, ofloxacin and nitrofurantoin respectively after the uropathogen was treated with 2120ug/ml dilution of thioridaxine. Whereas 112.5% loss of resistance was recorded for ciprofloxacin, 100% and 130% losses were recorded for chloramphenicol and nitrofurantoin respectively after 2160ug/ml thioridaxine treatment. After 2200ug/ml treatment, 60% loss of resistance was recorded for nitrofurantoin while less than 40% and 10% losses were recorded for ciprofloxacin and ofloxacin respectively. Fifty percent (50%), 50%, 50%, 58.1% and 70% resistance losses were recorded for gentamicin, ciprofloxacin, chloramphenicol, ofloxacin and nitrofurantoin respectively after 2240ug/ml thioridaxine treatment.

Table 3 is a summary of ≤50% loss of antibiotic resistance after 2000-2240ug/ml treatment with thioridaxine laboratory dilutions. With regard to Table1, only gentamicin, ciprofloxacin, chloramphenicol, ofloxacin and nitrofurantoin recorded ≤50% loss in resistance. Thioridaxine dilutions of 2040ug/ml, 2160ug/ml and 2240ug/ml recorded mean±S.E loss of resistance for all five antibiotics of 68.0±16.3% and 55.0±25.0% respectively. Less than 45%, 45%, 45% and 15% loss of resistance were effected by 2000, 2080, 2120 and 2200ug/ml laboratory dilutions of thioridaxine for all five antibiotics. All the dilutions put together recorded mean ±S.E loss of resistance of 76.8±23.1% and 81.4±19.6% for ciprofloxacin and nitrofurantoin respectively. Also, the combined effect of all seven laboratory dilutions of thioridaxine recorded less than 15%, 16% and 50% loss of resistance for gentamicin, ofloxacin and chloramphenicol respectively.

Table 3. *Summary of ≤50% loss of resistance as induced by thioridaxine dilutions treatment on MDR Escherichia coli uropathogen*

Thioridaxine dilutions	Antibiotics whose Resistance was reduced by ≤50.0%					
	GEN (%)	CPR (%)	OFL (%)	NIT (%)	CHL (%)	mean±S.E
2000ug/ml	0.0	0.0	0.0	90.0	115.0	41.0±24.4
2040ug/ml	0.0	250.0	0.0	50.0	50.0	68.0±24.4
2080ug/ml	50.0	50.0	0.0	50.0	50.0	40.0±11.8
2120ug/ml	0.0	75.0	50.0	80.0	0.0	41.0±13.2
2160ug/ml	0.0	112.5	0.0	130.0	100.0	68.5±16,3
2200ug/ml	0.0	0.0	0.0	6.0	0.0	12.0±5.7
2240ug/ml	50.0	50.0	58.1	70.0	50.0	55.6±25.0

Data on the effect of thioridaxine dilutions on the minimum inhibitory concentration (MIC) of chloramphenicol on the multidrug resistant *Escherichia coli* uropathogen are shown in Table 4. At the end of 24 hours incubation at 37^{o}C of the experimental set up, inoculum control tubes for all thioridaxine dilutions (2000-2240ug/ml) showed turbidity (cloudiness) as expected. As expected also, sterile broth control and drug control tubes remained clear at the end of incubation. Laboratory dilutions of 2000ug/ml, 2040ug/ml, 2120ug/ml and 2200ug/ml did not affect the minimum inhibitory concentration (MIC) of chloramphenicol as the MIC remained 30ug. Reductions in MIC of chloramphenicol were however effected by 2080ug/ml, 2160mg/ml and 2240ug/ml thioridaxine dilutions. Thioridaxine dilutions of 2080ug/ml and 2160ug/ml reduced chloramphenicol MIC to 15ug each which is a two-fold reduction, while 2240ug/ml reduced the MIC to 7.5ug (a four-fold reduction).

Table 4. *The effect of thioridaxine dilutions on the minimum inhibitory concentration (MIC) of chloramphenicol on a multidrug resistant Escherichia coli isolate obtained from mid-stream urine after 24hr incubation at 370C*

Thioridaxine Dilutions (ug/ml)	New MIC after Thioridaxine Treatment	Serial dilutions of chloramphenicol (ug)									
		120.0	60.0	30.0	15.0	7.50	3.75	1.88	Inoculum control	Sterile Broth control	Drug control
2000	No change	-	-	-	+	+	+	+	+	-	-
2040	No change	-	-	-	+	+	+	+	+	-	-
2080	15ug(2-fold)	-	-	-	-	+	+	+	+	-	-
2120	No change	-	-	-	+	+	+	+	+	-	-
2160	15ug(2-fold)	-	-	-	-	+	+	+	+	-	-
2200	No change	-	-	-	+	+	+	+	+	-	-
2240	7.5ug(4-fold)	-	-	-	-	-	+	+	+	-	-

4. Discussion

This study has the underlining intent of making suggestions aimed at reclaiming some common old and not too old drugs which are losing therapeutic usefulness owing to ineffectiveness in terms of therapeutic outcome. The alternative is to replace the old drugs with new ones but it will be counter-productive because such new drugs may be more costly, may be toxic (i.e. may have more adverse side effects), their use may need much longer stay in the hospital (or longer duration of treatment) and their use may require treatment in intensive care units.

Antibiotic susceptibility profiles of all five strains (uropathogens) of *Escherichia coli* before thioridaxine treatment in this study showed that the five uropathogen strains were sensitive to gentamicin, ciprofloxacin, chloramphenicol, ofloxacin and nitrofurantoin with mean± SE zones of inhibition of 10. 6 ± 7.2mm, 10.2 ± 8.8mm, 8.2 ± 5.6mm, 15.6 ± 20.1mm and 9.6 ± 11.3mm respectively. The implication of this is that 5(55.6%) of the antibiotics recorded positive reactions at the end of incubation. Whereas the five uropathogen strains were resistant to augmentin, strain EC_2 resisted cefuroxime, nalidixic acid and tetracycline. Strains EC_1 and EC_4 resisted cefuroxime also. The fact that EC_2 strain resisted three antibiotics qualifies it as a multidrug organism (Jan *et al*., 2004; Otajevwo, 2012). Findings suggest that infections or diseases caused by MDR *Escherichia coli* strain 2 in the study environment and perhaps in other environments can be treated successfully with gentamicin/ciprofloxacin/ofloxacin (i.e. any one of the three) only or in synergistic combination with either chloramphenicol or nitrofurantoin or any other combination a physician may consider safe and potent. The privilege of choosing any of chloramphenicol, gentamicin or nitrofurantoin as alternative to ciprofloxacin or ofloxacin (both fluoroquinolones) will be cheering to low income patients in terms of availability. The total resistance recorded against augmentin is worrisome because it is a drug that is used to treat a good number of human diseases. Some authors have also expressed similar worry over augmentin in terms of antibiotic susceptibility (Oluremi *et al*., 2011*;* Otajevwo, 2012; Otajevwo, 2014). It was not clear as to whether the site from where the pathogens were isolated had any direct or indirect effect on the antibiograms of the strains as recorded in this study. However, it may be possible that pH changes or variation from site and presence /absence of oxygen could affect the response of *Escherichia coli* (a facultative aerobe) to relevant antibiotics it is exposed to invitro. The sensitivity profile obtained in this study however, is subject to verification and confirmation by other researchers.

The fact that each of the five strains was resistant to one-three of the antibiotics used in this study may suggest that very large population of *Escherichia coli* organisms have been exposed to several antibiotics (Oluremi *et al*., 2011). Thioridaxine laboratory dilutions of 2000ug/ml, 2040ug/ml, 2080ug/ml, 2120ug/ml, 2160ug/ml, 2200ug/ml and 2240ug/ml were used to treat and cure five uropathogenic strains of *Escherichia coli* with the intent of reducing their resistance significantly or eliminating it completely. The loss of 50-100% (≤50%) of resistance after treatment with the stated dilutions of thioridaxine was used as the basis of establishing the curing effects of these dilutions. The use of 50% and above loss in resistance as a criterion to determine the extent of plasmid curing was according to the scheme provided by Akortha *et al*. (2011). Stanier *et al*. (1984) reported that the elimination of plasmids by dyes and other natural agents reflects the ability of such an agent to inhibit plasmid replication at a concentration that does not affect the chromosome.

After treatment with 2000-2240ug/ml thioridaxine dilutions, *Escherichia coli* strain 2 still remained completely resistant to nalidixic acid, cefuroxime, tetracycline and augmentin. This could be due to the fact that plasmids responsible for resistance to these drugs (present in the genome of the uropathogen) may be chromosome- mediated or non- conjugative plasmids (Akortha and Filgona, 2009). Each of the seven laboratory dilutions induced ≤50.0% loss of resistance of the uropathogen to nitrofurantoin (Table 2). Thioridaxine dilution of 2000ug/ml induced 115% and 90% loss of resistance of the uropathogen to chloramphenicol and nitrofurantoin respectively. This represented 2(22.2%) of the total selected drugs used in this study. Also, only two antibiotics namely ciprofloxacin and nitrofurantoin recorded 250% and 90% resistance losses respectively after 2040ug/ml thioridaxine treatment. In a similar study, some authors have used 2000ug/ml thioridaxine dilution treatment to induce loss of resistance (enhance antibiotic sensitivity) in some multidrug resistant strains of *Pseudomonas aeruginosa* and recorded elimination (curing) of antibiotic resistance in thioridaxine treated strains (Mukherjee *et al*., 2012). It was concluded in their report that the antipsychotic drug-thioridaxine is a potent agent able to eliminate drug resistance plasmids which are much longer in size than the plasmids of other gram negative bacteria (Mukherjee *et al*., 2012). Thioridaxine dilution of 2080ug/ml induced ≤50% resistance loss to gentamicin, ciprofloxacin, chloramphenicol and nitrofurantoin. Whereas mean±S.E percentage loss of resistance after 2040ug/ml treatment of the uropathogen was 68.0±24.4%, 2080ug/ml thioridaxine treatment recorded 40.0±11.8 (less than 50%) loss of resistance (Table 3). Resistance losses of 75%, 50% and 80% for ciprofloxacin, ofloxacin and nitrofurantoin respectively were recorded after 2120ug/ml thioridaxine treatment with a corresponding mean±S.E resistance loss of 41.0±13.2% (which is less than 50%). Laboratory thioridaxine dilutions of 2160ug/ml and 2240ug/ml effected 112.5%, 100% and 130% resistance losses for ciprofloxacin, chloramphenicol and nitrofurantoin respectively for the first dilution and 50%, 50%, 50%, 58.1% and 70% resistance losses for gentamicin, ciprofloxacin, chloramphenicol, ofloxacin and nitrofurantoin respectively for the second dilution. Both dilutions however, recorded corresponding mean±S.E resistance losses of 68.5±16.3%

and 55.6±25.0% respectively.

The lowest resistance loss was recorded by 2200ug/ml which recorded more than 50% resistance loss for nitrofurantoin only with a corresponding 12.0±5.7%. From these results, it seems any of thioridaxine dilutions of 2160ug/ml or 2040ug/ml or 2240ug/ml may possess the capacity to eliminate resistance put up by multidrug resistant strains of *E.coli* and therefore, could be administered in the therapeutic control of various infections caused by such bacteria. According to Mukherjee *et al.* (2012), the simultaneous application of thioridaxine to patients may open up a new arena of therapy. The simultaneous application of thioridaxine may not only act as an additional antibacterial agent but may also help to eliminate the drug resistant plasmids from the infectious bacterial cells (Spendler *et al*., 2006). Hence patients suffering from MDR *Escherichia coli* infections may be administered thioridaxine at standard human doses (using 2160ug/ml or 2040ug/ml or 2240ug/ml as a basis) along with antibiotics especially gentamicin, chloramphenicol, nitrofurantoin or any of the fluoroquinolones (ciprofloxacin, ofloxacin).

Also in this study, sensitivity enhancement effect of thioridaxine laboratory dilutions on the minimum inhibitory concentration (MIC) of chloramphenicol as it affected MDR *E.coli* strain 2 uropathogen showed a two-fold (15ug), two-fold (15ug) and four fold (7.5ug) reductions in MIC of chloramphenicol as recorded for 2080ug/ml, 2160ug/ml and 2240ug/ml thioridaxine dilutions respectively. Some authors had reported similar findings on MDR *Staphylococcus aureus* strains (Otajevwo and Momoh, 2013) as well as on MDR strains of *Pseudomonas aeruginosa* using acridine orange (Otajevwo and Okungbowa, 2014). Otajevwo (2012) reported similar results using ethidium bromide dilutions on MDR strain of *E.coli*. A fast and accurate determination of MIC can ensure optimal effective treatment of patients while at the same time avoiding over-prescription. This will save money for healthcare providers as well as reduce development of resistance (NCCLS, 2000; McGowan and Wise, 2001).

The MIC of chloramphenicol which is 30ug (based on long standing research) was reduced to 15ug (two fold reduction), 15ug (two fold reduction) and 7.5ug (four fold reduction) by thioridaxine laboratory dilutions of 2080ug/ml, 2160ug/ml and 2240ug/ml respectively as tested on multiple resistant drug strain of *Escherichia coli* isolated from the urinary tract of a patient. According to Dimitru *et al*. (2006), there is a significant correlation between MIC values and the inhibition zone diameters obtained by a 30ug disc. The lower the MIC and the larger the zone of inhibition, the more susceptible the microorganism is to the antimicrobial agent and conversely, the higher the MIC and smaller the zone of inhibition, the more resistant the microorganism (Dimitru *et al*., 2006).

The medical implication therefore of the two-fold, two-fold and four-fold reduction of chloramphenicol MIC by thioridaxine dilutions of 2080ug/ml, 2160ug/ml and 2240ug/ml respectively is that when doses of one of these dilutions or a combination of any two are incorporated into the manufacture of chloramphenicol or any other related antibiotic and then administered to a patient diagnosed to be suffering from a disease caused by MDR *E.coli* strain, a better result in terms of outcome (cure of the disease) may be achieved as it will require four times its concentration to function invivo. In a related work, Kohler (2010) showed that the resistance of *P. aeruginosa* to tetracycline efflux was reduced from MIC 0.032 to 0.004ug/ml (an eight- fold reduction) by treatment with phenothiazine. Crowle *et al*. (1992) demonstrated that non- toxic concentrations of phenothiazine in the lungs achieved complete elimination of *Mycobacterium tubercolosis*. In a related study, some workers had reported the capacity of an aqueous methanolic plant- extract- epidiosbulbin- E- Acetate (EEA) to decrease the MIC of antibiotics against MDR bacteria thus making antibiotic treatment more effective (Shiram *et al*., 2008)

5. Conclusion

Thioridaxine laboratory dilutions of 2040ug/ml, 2160ug/ml and 2240ug/ml induced more than 50% resistance losses for gentamicin, ciprofloxacin, ofloxacin, nitrofurantoin and chloramphenicol. Also, 2080ug/ml, 2160ug/ml and 2240ug/ml thioridaxine dilutions effected chloramphenicol MIC reductions by two-fold, two-fold and four-fold respectively. Hence, patients suffering from MDR *Escherichia coli* infections may be administered thioridaxine at standard human doses (using 2160ug/ml and or 2240ug/ml as a basis) along with any of the above antibiotics (singly or in combination). It is hoped that this simultaneous or part application of thioridaxine with antibiotics will eliminate plasmids while enhancing the penetration of antibiotics into pathogenic bacterial cells especially those of MDR *E.coli.* The overall effect of this is that patient recovery will be facilitated and hospital stay and hospital cost would be drastically reduced.

Suggestions for Further Studies

The determination of resistance loss by multidrug resistant *E.coli* strain 2 after treatment with 2080ug/ml, 2160ug/ml and 2240ug/ml thioridaxine dilutions may not be totally convincing. It is recommended therefore, that the effects of these dilutions in the pathogen and indeed, other commonly known pathogens at the molecular level be probed further by carrying out plasmid profiling before and after treatment to see at what dilution(s) there is partial or total elimination of drug resistance “R” plasmid bands.

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