academicJournals

Vol. 6(1), pp. 24-33, January 2014
DOI: 10.5897/IJMMS2013.0967
ISSN 2006-9723 ©2014 Academic Journals
http://www.academicjournals.org/IJMMS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

A study on resistance loss of multidrug resistant (MDR) *Pseudomonas aeruginosa* strains after treatment with dilutions of acridine orange

Otajevwo F. D. and Okungbowa A.

Department of Microbiology and Biotechnology, Western Delta University, Oghara, Delta State, Nigeria.

Accepted 7 October, 2013

This study investigated the loss of resistance of multidrug resistant (MDR) *Pseudomonas aeruginosa* strains after exposure to dilutions of acridine orange. Five pure axenic strains of *P. aeruginosa* coded PA_1 to PA_5 obtained from five infected human sources which included middle ear, urethra, trachea, wound and urine were obtained from the Medical Microbiology Department of the University of Benin Teaching Hospital, Nigeria and stocked on sterile Nutrient agar slants. Slant cultures were sub-cultured aseptically on sterile MacConkey and Blood agar plates and incubated aerobically at 37°C for 24 h to confirm for *P. aeruginosa.* Gram staining and oxidase test were carried out on resulting colonies. Antibiotic sensitivity test was done by agar disc diffusion method on all confirmed strains on sterile Mueller-Hinton agar plates before and after treatment with acridine orange (AO). *P. aeruginosa* strains that showed ≤50.0% reduction in resistance markers (RM) after treatment with 0.35, 0.55, 0.75 and 0.95 µg/ml dilutions of AO were noted. Minimum inhibitory concentration (MIC) assay was done using gentamicin on PA_5 strain with all four dilutions. All five strains showed 100% resistance against augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. Sensitivity was recorded for ofloxacin and gentamicin with 14.6±9.5 and 8.4±4.4 mm zones of inhibition, respectively for all the strains except strain PA_3 which was resistant to 8 (100.0%) of antibiotics used. Strains PA_1, PA_2, PA_4 and PA_5 were each resistant to 6 (75.0%) of the antibiotics tested. There was loss of RM of 52.1±18.6 and 54.7±37.6% to ofloxacin after treatment with 0.35 and 0.55 µg/ml dilutions, respectively by all MDR *P. aeruginosa* strains. Loss of RM to gentamicin by strains PA_1, PA_2, PA_4 and PA_5 after 0.35 µg/ml acridine orange treatment was recorded as 0.0, 61.5, 58.3 and 60.0%, respectively with a mean±standard error (SE) of 45.0±15.0%. With 0.55 µg/ml dilution, 97.6±28.3% loss of RM was recorded while less than 45.0 and 35.0% loss of RM were recorded for 0.75 and 0.95 µg/ml dilutions, respectively. Acridine orange dilutions of 0.35 and 0.55 µg/ml recorded two-fold (5 µg) and four-fold (2.5 µg) reduction in MIC of gentamicin, respectively. The implications of these findings are discussed.

Key words: Resistance loss, Pseudomonas aeruginosa, treatment, dilutions, acridine orange

INTRODUCTION

Pseudomonas aeruginosa is a highly invasive and toxigenic aerobic Gram negative bacterium. It is non-sporing, non-capsulated and usually motile with the help of one or two flagella. The organism readily grows over a wide range of temperature and media. The reasons for the preeminence of this microorganism as a human pathogen range from its adaptability, its innate resistance to many antibiotics, disinfectants and its virulence factors (Aendekerk et al., 2005). The organism is a danger and threat to patients with cystic fibrosis or AIDS and other

*Corresponding author. E-mail: fesdotaj@yahoo.com or dafotaj@hotmail.com.

immune disorders as well as those requiring long-term hospitalization. *P. aeruginosa* is an opportunistic pathogen with innate resistance to many antibiotics and disinfectants (Shahid et al., 2003). The microorganism is physiologically versatile and flourishes as a saprophyte in multiple environments including sinks, drains, respirators, humidifiers, and disinfectant solutions (Govan and Deretic 1996). *P. aeruginosa* is notorious for its resistance to antibiotics as it maintains antibiotic resistance plasmid (R) factor (Radi and Rahman, 2010). These plasmids are transmissible to sensitive bacteria which make them acquire resistance to antibiotics and have the ability to undergo recombination through conjugation, transformation and transduction. Multidrug active efflux systems have recently been recognized in a number of bacteria as efficient mechanisms of resistances in *P. aeruginosa* by which antibiotics are expelled from the cells by membrane transporter proteins, the so called drug efflux pumps (Lomovskaya et al., 2001).

Infections due to *P. aeruginosa* are seldom encountered in healthy adults. The organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients especially those with impaired immune defenses (Neu, 1993). The indiscriminate use of antimicrobial drugs parti-cularly in hospitalized patients leads to the suppression of drug susceptible organisms in the gut flora and favors the persistence and growth of resistant bacteria including *P. aeruginosa*. The closed environment of hospitals favors the transmission of such resistant strains through personnel, fomites and by direct contact (Melnick and Adelberg, 1998).

P. aeruginosa is the most common and lethal pathogen responsible for urinary tract infections (UTI), ventilator associated pneumonia in intubated patients with directly attributable deaths reaching 38% (Mansouri et al., 2011). Multidrug resistant (MDR) *P. aeruginosa* is found to be resistant to a very large number of antibiotics (Bonomo and Szabo, 2006; Manikandan et al., 2011). Resistance may be due to interplay of various interactions including beta-lactamases, mutations, decreased permeability and the activities of efflux pumps (Defez et al., 2004; Abdi-Ali et al., 2007) and presence of drug resistant plasmids (Ranjbar et al., 2007).

P. aeruginosa is a serious threat in clinical medicine since most isolates are resistant simultaneously to many antibiotics at very high levels (Mukherjee et al., 2011). Elimination of these resistance markers (RM) by known pharmacological compounds would be advantageous in successful therapeutic control of various infections caused by this pathogen (Mukherjee et al., 2011).

There are a number of reports demonstrating the ability of various chemical and physical agents to increase the rate of loss of plasmid DNA from bacteria (Sonstein and Baldwin, 1972; Stanier, 1984; Otajevwo, 2012). Antibiotics such as mitomycin, rifampicin, novobiocin and flavophospholipol as well as DNA intercalating dyes (such (such as acridine orange, ethidium bromide, acriflavine and ascorbic acid) have been shown to cure many plasmids (Ramesh et al., 2000). Acridine orange has been shown to cure F-plasmids from *Escherichia coli* and it is suggested that this dye interfers with plasmid replication, stimulating the entire plasmid loss (Salisbury et al., 1972).

It has also been reported that acridine orange and ethidium bromide are better curing agents for *Pseudomonas cepacia* than sodium dodecyl sulphate (SDS) and elevated temperature (Kumar and Surendran, 2006). Stanier (1984) reported that the elimination of plasmids by dyes and other agents reflects the ability of such agents to inhibit plasmid replication at a concentration that does not affect the chromosome. Acridine orange, ethidium bromide, mitomycin and SDS failed to cure the plasmid of *Pseudomonas putida*, though the phenotypic characteristics changed and the plasmid was cured at a frequency of 2.63% when acridine orange and elevated temperature (40°C) were used together (Stanier, 1984).

Ingram et al. (1972) found that drug resistance of *P. aeruginosa* could be eliminated by treatment with SDS and Pattnakik et al. (1995) reported that acridine orange could not affect *P. aeruginosa* due to impermeability of its cell wall while ethidium bromide and SDS cured antibiotic resistance plasmid at a concentration of 1 to 2% and 700 to 3000 µg/ml for SDS and ethidium bromide, respectively. Al-Amar et al. (1999) treated an isolate of *P. aeruginosa* with 1000 µg/ml dilution of acridine orange and reported that there were no cured cells as the plasmid profile of cured cells was the same as that of untreated samples with a conclusion that acridine orange had no effect on *P. aeruginosa* as a curing agent.

Treatments that increase frequency of elimination of plasmids will certainly enhance sensitivity (effectiveness) of antibiotics *in situ*. There is dearth of literature on antibiotic sensitivity improvement (through loss of RM) using dilutions of chemical agents such as acridine orange. Hence, the aim of this work was to study the RM loss of MDR *P. aeruginosa* strains after treatment with clinical dilutions of acridine orange with the following objectives:

(1) Determine the sensitivity profile of *P. aeruginosa* strains (from selected diseased sites of the human body) before treatment with dilutions of acridine orange after incubation for 24 h at 37°C.
(2) Determine the distribution of multidrug RM among the selected pathogenic strains of *P. aeruginosa*.
(3) Determine *P. aeruginosa* strains that showed ≤50.0% loss of their RM after 0.35 µg/ml acridine orange dilution treatment and incubation at 37°C for 24 h.
(4) Determine *P. aeruginosa* strains that showed ≤50.0% loss of their RM after 0.55, 0.75 and 0.95 µg/ml acridine orange dilution treatments and incubation at 37°C for 24 h.
(5) Determine the distribution of ≤50.0% loss of RM among MDR *P. aeruginosa* strains after treatment with

0.35, 0.55, 0.75 and 0.95 µg/ml dilutions of acridine orange.
(6) Determine acridine orange dilutions' effect(s) on the minimum inhibitory concentration (MIC) of gentamicin (an aminoglycoside) on an MDR *P. aeruginosa* strain from midstream urine.

MATERIALS AND METHODS

Sampling

Five pure axenic strains of *P. aeruginosa* coded PA_1 to PA_5 were obtained from the Medical Microbiology Department of University of Benin Teaching Hospital, Edo State, Nigeria. Bacterial pathogens which were isolated from ear swab, urethral swab, tracheal (throat) swab, wound swab, and mid stream urine samples, respectively, were inoculated aseptically (in pure form), on sterile nutrient agar slants and incubated at 37°C for 24 h. All strains were appropriately labeled.

Processing of samples

Colonies from resulting stocked cultures were re-confirmed by sub-culturing them on sterile nutrient agar (LabM, UK), MacConkey agar (LabM, UK) and Blood agar plates and inoculated plates were incubated at 37°C for 24 h. Purity test was done on all prepared agar plates before use to guarantee their sterility. Resulting colonies were inoculated on the selective medium centrimide agar and incubated appropriately.

Finally, oxidase test and gram staining were done on resulting colonies according to scheme provided by Cowan and Steel (1993). Colonies were stocked on sterile nutrient agar slants and kept at 4°C in the refrigerator for further use. The set up was appropriately labeled. All isolated bacterial pathogens were subjected to antibiotics sensitivity testing before treatment with dilutions of acridine orange.

Antibiotic sensitivity testing

Antibiotic sensitivity testing on each of the five pure strains of *P. aeruginosa* was carried out using the disc diffusion method on sterile Mueller-Hinton agar (MHA) plates (Bauer et al., 1966). A loop full of each colony was picked aseptically using a flamed wire loop and placed in the centre of 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 min to allow the agar surface to dry. A pair of forceps was flamed and cooled and used to pick antibiotic multidisc (Abitek, Liverpool) containing augmentin (30 µg), ofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (200 µg), cotrimoxazole (25 µg), amoxicillin (25 µg) and tetracycline (25 µg). Discs were placed at least 22.0 mm from each other and 14.0 mm from the edge of the plate (Ochei and Kolhatkar, 2008). Antibiotics discs were selected on the basis of their clinical importance and efficacy on *P. aeruginosa*. Plates were allowed to stand for 10 min tunes before incubation (Mbata, 2007).

The plates were incubated at 37°C for 24 h. A reference control strain of *P. aeruginosa* NCTC was inoculated in the same way on another plate with the same antibiotic discs and incubated at the same temperature and time. At the end of incubation, the diameters of the zones of inhibition from one edge to the opposite edge 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., 2002) and were noted and used further.

Preparation of dilutions of acridine orange

For treatment of MDR *P. aeruginosa* strains, sub-clinical acridine orange concentrations of 0.35, 0.55, 0.75 and 0.95 µg/ml were used. These concentrations (dilutions) were chosen in line with non-toxic laboratory concentrations of 0.25 to 1.0 µg/ml prescribed by Wurmb-Schwark et al. (2006) for ethidium bromide which have been reported to possess curing potentials. Hence, acridine orange dilutions of 0.35, 0.55, 0.75 and 0.95 µg/ml were prepared using RV/O where stock or original concentration of acridine orange used was 1.0 mg/ml or 1000 µg/ml. To obtain 0.35 µg/ml dilution therefore, 0.14 ml of stock reagent was added to sterile 9.86 ml of Mueller-Hinton broth. To obtain 0.55 µg/ml acridine orange dilution, 0.11 ml stock acridine orange solution was added to sterile 19.78 ml Mueller-Hinton broth. Also to obtain 0.75 µg/ml dilution, 0.30 ml stock solution was added to sterile 19.70 ml Mueller-Hinton broth and 0.38 ml stock solution of acridine orange was added to sterile 19.61 ml of Mueller-Hinton broth to obtain 0.95 µg/ml dilution. All dilutions were effected in sterile universal bottles containing sterile 20.0 ml of Mueller-Hinton broth each.

Growing broth culture of MDR *P. aeruginosa* strains

A colony of each MDR strain was aseptically picked from its slant stock culture using flamed and cooled wire loop and inoculated into sterile 10 ml Mueller-Hinton broth. Inoculated broths were incubated at 37°C for 18 h. The resulting turbid broth culture was then diluted according to a modified method of Shirtliff et al. (2006). Using a sterile pipette, 0.1 ml of broth culture was mixed with 99.9 ml (1:200 dilution) of sterile Mueller-Hinton broth. This was properly mixed and was used as working inoculum and should contain 10^5 to 10^6 organisms, which was used within 30 min (Ochei and Kolhatkar, 2008).

Treatment of MDR pathogenic strains with prepared acridine orange dilutions

The treatment of MDR *P. aeruginosa* pathogenic strains with the prepared dilutions of acridine orange was done according to a modified method of Byron et al. (2003). Using a sterile pasteur pipette, 0.5 ml aliquot of each diluted overnight broth culture of MDR pathogen was added to 4.5 ml sterile molten Nutrient agar. The various prepared dilutions (one at a time) of acridine orange were then added in 0.5 ml volume. The set up was properly mixed and labeled. 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 multidisc 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°C for 24 h. Measurement of diameters of zones of inhibition was taken and recorded (NCCLS, 2000).

Determination of MIC of gentamicin after acridine orange treatment

Serial doubling dilutions of gentamicin, the antibiotic to which a *P. aeruginosa* strain (isolated from mid stream urine) recorded more than 50.0% RM loss was carried out. The antibiotic being used for this assay was sterilized by filtration before use. Sterile test tubes, numbering 13 were set up on a test tube rack and labeled 1 to13.

Using a sterile pipette, 1 ml of diluted broth was dispensed into tubes 2 to 10, 11 and 13. Into tube 12, 2.0 ml of diluted broth culture was pipetted. Tube 11 was the inoculum control, tube 12 was the broth control and 13 was the drug control. Into tubes 1, 2 and 13, 1 ml of the working antibiotic solution was pipetted. Serial doubling dilutions of antibiotic solution were separately prepared in

Table 1. Sensitivity profile of *P. aeruginosa* strains before treatment with subclinical concentrations of acridine orange after incubation at 37°C for 24 h.

Strain code	Zone of inhibition (mm)							
	AUG	OFL	GEN	NAL	NIT	COT	AMX	TET
PA_1 (Ear swab)	0.0	18.0	13.0	0.0	0.0	0.0	0.0	0.0
PA_2 (Urethral swab)	0.0	16.0	12.0	0.0	0.0	0.0	0.0	0.0
PA_3 (Tracheal swab)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PA_4 (Wound swab)	0.0	20.0	12.0	0.0	0.0	0.0	0.0	0.0
PA_5 (Urine)	0.0	19.0	5.0	0.0	0.0	0.0	0.0	0.0
Mean±SE zone of inhibition	0.0	14.6±9.54	8.4±4.40	0.0	0.0	0.0	0.0	0.0

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

Table 2. Distribution of multi drug resistance markers among pathogenic strains of *P. aeruginosa.*

Strain code	3 Drugs	4 Drugs	5 Drugs	6 Drugs	< Drugs	Drugs resisted
PA_1 (Ear swab)	-	-	-	+	-	Augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline
PA_2 (Urethral swab)	-	-	-	+	-	Augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline
PA_3 (Tracheal swab)	-	-	-	-	+	Augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin tetracycline, ofloxacin and gentamicin
PA_4 (Wound swab)	-	-	-	+	-	Augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline
PA_5 (Urine)	-	-	-	+	-	Augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline

nutrient broth to get reducing concentrations of the antibiotic using 100 to 0.4 µg/ml as a standard for MIC assay (Ochei and Kolhatkar, 2008). All tubes were incubated at 37°C for 18 h. Turbidity (cloudiness) in the growth medium indicated growth. Tube 11 showed turbidity and tubes 12 and 13 showed no growth. The lowest concentration showing no growth was the MIC of the antimicrobial agent as effective against *P. aeruginosa* strains. All MIC results before and after treatment with dilutions of acridine orange were recorded accordingly.

RESULTS

Table 1 shows *P. aeruginosa* strains PA_1, PA_2, PA_3, PA_4, and PA_5 isolated from ear swab, urethral swab, tracheal swab, wound swab and midstream urine, respectively and their sensitivity reactions to augmentin, ofloxacin, gentamicin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. All five strains showed 100.0% resistance against augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. Sensitivity was recorded for oflaxacin and gentamicin with mean±standard error (SE) zones of inhibition of 14.6±9.54 and 8.4±4.40 mm, respectively for all *P. aeruginosa* strains with exception of PA_3 which did not respond to the tested drugs at all.

The occurrence of multidrug resistance *P. aeruginosa* strains after antibiotic susceptibility testing is shown in Table 2.

Strains PA_1, PA_2, PA_4 and PA_5 isolated from ear swab, urethra swab, wound swab and mid stream urine, respectively were each resistant to 6 (75.0%) of the total antibiotics tested. Interestingly, all four *P. aeruginosa* strains were each sensitive to ofloxacin and gentamicin. Only strain PA_3 (isolated from tracheal swab) resisted 8 (100.0%) or the entire antibiotics used.

In Table 3, the percentage loss of RM due to 0.33 µg/ml acridine orange curing effect is shown. Loss of 50% and above of RM was recorded for *P. aeruginosa* strains PA_1 (with 78.5% loss of resistance to ofloxacin), PA_2 (with 80.0 and 61.5% loss of resistance to ofloxacin and gentamicin, respectively), PA_4 (with 50.0 and 58.3% loss of

Table 3. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance biomarkers after treatment with 0.35 µg/ml dilution of acridine orange and incubation at 37°C for 24 h.

MDR strain		Zone of inhibition (mm)							
		AUG (%)	OFL (%)	GEN (%)	NAL (%)	NIT (%)	COT (%)	AMX (%)	TET (%)
PA_1	Before	0.0	14.0	14.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	25.0 (78.5)	19.0 (35.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_2	Before	0.0	15.0	13.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	27.0 (80.0)	21.0 (61.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_3	Before	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_4	Before	0.0	16.0	12.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	26.0 (50.0)	19.0 (58.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_5	Before	0.0	19.0	5.0	17.0	0.0	0.0	12.0	0.0
	After	0.0 (0.0)	25.0 (31.5)	8.0 (60.0)	21.0 (23.5)	0.0 (0.0)	0.0 (0.0)	14.0 (16.6)	0.0 (0.0)

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

resistance to ofloxacin and gentamicin, respectively). PA_5 strain recorded 60.0% loss of resistance to gentamicin. With the exception of strain PA_3, all strains maintained 100.0% resistance to 6 (75.0%) of the antibiotics used which included: augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline after treatment with 0.35 µg/ml acridine orange. Strain PA_3 resisted 8 (100.0%) of the antibiotics used.

Table 4 shows percentage loss of RM due to 0.55 µg/ml curing effect of acridine orange. Loss of 50% and above of RM was recorded for *P. aeruginosa* strains PA_1 (with 50.0% loss to gentamicin), PA_2 (with 160.0 and 76.9% loss to ofloxacin and gentamicin respectively), PA_4 (with 83.3% loss to gentamicin) and PA_5 with 58.8 and 180.0% loss of RM of ofloxacin and gentamicin, respectively. Strain PA_3 maintained 100.0% resistance to 8 (100.0%) of the antibiotics used while the remaining four strains maintained 100.0% resistance to 6 (75.0%) of the antibiotics used after treatment with 0.55 µg/ml acridine orange.

Data on 50% and above reduction in RM in *P. aeruginosa* strains after treatment with 0.75 µg/ml acridine orange are shown in Table 5. *P. aeruginosa* strains PA_1 and PA_5 recorded 50 and 120.0% loss, respectively in RM to gentamicin. Strain PA_2 recorded less than 35.0% loss in RM to ofloxacin and gentamicin. Strains PA_4 and PA_5 each recorded less than 20% loss in resistance to ofloxacin. *P. aeruginosa* strain PA_3 showed 100.0% resistance to 8 (100.0) of drugs used, while other strains recorded 100.0% resistance to augmentin and tetracycline. There were slight losses in RM for nalidixic acid, nitrofurantoin, cotrimoxazole and amoxicillin as recorded for PA_1, PA_4 and PA_5.

The effect of 0.95 µg/ml acridine orange treatment of the *P. aeruginosa* strains on the selected antibiotics is shown in Table 6. Only *P. aeruginosa* strain PA_5 recorded 120.0% loss in RM gentamicin. Strain PA_1 showed less than 25.0% loss of ofloxacin and gentamicin. Strain PA_2 recorded less than 10.0% and less 40.0% loss of ofloxacin and gentamicin respectively. Strain PA_4 showed less than 10.0% loss to gentamicin while PA_5 recorded 120.0% loss in RM to gentamicin. Strains PA_1, PA_2, PA_4 and PA_5 recorded 100.0% resistance loss to 6 (75.0%) of the antibiotics used after acridine orange treatment, while strain PA_3 showed 100.0% loss to 8 (100.0%) of the drugs used for the susceptibility testing.

Presented in Table 7, is the effect of subclinical dilutions of acridine orange on the MIC of gentamicin as it inhibited the growth of the growth of

Table 4. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance markers after treatment with 0.55 µg/ml dilution of acridine orange and incubation at 37°C for 24 h.

MDR strain		Zone of Inhibition (mm)							
		AUG (%)	OFL (%)	GEN (%)	NAL (%)	NIT (%)	COT (%)	AMX (%)	TET (%)
PA_1	Before	0.0	14.0	14.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	18.0 (28.5)	21.0 (50.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_2	Before	0.0	10.0	13.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	26.0 (160.0)	23.0 (76.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_3	Before	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_4	Before	0.0	20.0	12.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	24.0 (20.0)	22.0 (83.3)	0.0 (0.0)	0.0 (0.0)	0.0 0.0)	0.0 (0.0)	0.0 (0.0)
PA_5	Before	0.0	17.0	5.0	17.0	0.0	0.0	12.0	0.0
	After	0.0 (0.0)	27.0 (58.8)	14.0 (180.0)	21.0 (23.5)	0.0 (0.0)	0.0 (0.0)	14.0 (16.6)	0.0 (0.0)

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

Table 5. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance markers after treatment with **0.75 µg/ml** dilution of acridine orange and incubation at 37°C for 24 h.

MDR strain		Zone of inhibition (mm)							
		AUG (%)	OFL (%)	GEN (%)	NAL (%)	NIT (%)	COT (%)	AMX (%)	TET (%)
PA_1	Before	0.0	14.0	14.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	18.0 (28.5)	21.0 (50.0)	8.0 (0.0)	0.0 (0.0)	11.0 (0.0)	6.0 (0.0)	0.0 (0.0)
PA_2	Before	0.0	20.0	13.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	26.0 (30.0)	17.0 (30.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_3	Before	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_4	Before	0.0	20.0	12.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	23.0 (15.0)	12.0 (0.0)	19.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_5	Before	0.0	19.0	5.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	21.0 (10.5)	11.0 (120.0)	0.0 (0.0)	13.0 (0.0)	0.0 (0.0)	0.0(0.0)	0.0 (0.0)

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

MDR *P. aeruginosa* strain PA_5 (isolated from midstream urine) or the effect of the dilutions in RM reduction or otherwise, of PA_5 strain. Whereas 0.35 µg/ml acridine orange dilution caused a two-fold (5.0 µg) reduction in gentamicin MIC (which is 10 µg), 0.55 µg/ml dilution caused a four-fold (2.5 µg) reduction. Both 0.75 and 0.95 µg/ml acridine orange dilutions recorded no effect (no change) in the MIC of gentamicin.

In Table 8, the summary and distribution of 50.0% and above loss in RM is presented. *P. aeruginosa* strains PA_2 and PA_5 recorded 160.0 and 58.8% loss in RM, respectively to ofloxacin with mean loss of 54.7±37.6% after treatment with 055 µg/ml acridine orange. This was followed by mean loss of 52.1±18.6% to ofloxacin after treatment with 0.35 µg/ml acridine orange. Strains PA_1, PA_2, PA_4 and PA_5 recorded 50.0, 76.9, 83.3 and 180.0% loss in RM, respectively to gentamicin with mean loss of 97.6±28.3% after treatment with 0.55 µg/ml acridine orange

Table 6. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance markers after treatment with **0.95 µg/ml** dilution of acridine orange and incubation at 37°C for 24 h.

MDR strain		Zone of Inhibition (mm)							
		AUG (%)	OFL (%)	GEN (%)	NAL (%)	NIT (%)	COT (%)	AMX (%)	TET (%)
PA_1	Before	0.0	14.0	14.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	17.0 (21.4)	16.0 (14.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_2	Before	0.0	20.0	13.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	21.0 (5.0)	18.0 (38.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_3	Before	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_4	Before	0.0	20.0	12.0	0.0	0.0	0.0	0.0	0.0
	After	0.0 (0.0)	20.0 (0.0)	13.0 (8.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PA_5	Before	0.0	19.0	5.0	17.0	0.0	0.0	12.0	0.0
	After	0.0 (0.0)	26.0 (36.8)	11.0 (120.0)	21.0 (23.5)	0.0 (0.0)	0.0 (0.0)	14.0 (16.6)	0.0 (0.0)

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

Table 7. The effect of acridine orange dilutions on the minimum inhibitory concentration (MIC) of gentamicin on a MDR *P. aeruginosa* strain isolated from mid stream urine after 24 h incubation at 37°C.

Acridine orange dilution (µg/ml)	New MIC after AO treatment (µg)	Serial dilutions of gentamicin (µg)											
		80.0	40.0	20.0	10.0	5.0	2.5	1.25	0.63	0.33	Inoculum control	Sterile broth control	Drug control
0.35	5.0 (2-fold)	-	-	-	-	-	+	+	+	+	+	-	-
0.55	2.5 (4-fold)	-	-	-	-	-	-	+	+	+	+	-	-
0.75	No change	-	-	-	-	+	+	+	+	+	+	-	-
0.95	No change	-	-	-	-	+	+	+	+	+	+	-	-

Gentamicin was chosen for MIC Assay because it was one of the two antibiotics that recorded ≤50.0% reduction in resistance markers. PA_5 (*P. aeruginosa* isolated from midstream urine) was selected and used for MIC assay on the basis of organism that showed up to ≤50.0% reduction in resistance markers for all four dilutions.

Table 8. Distribution of ≤50.0% loss of resistance markers among multidrug resistant *P. aeruginosa* strains after treatment with 0.35, 0.55, 0.75 and 0.95 µg/ml dilutions of acridine orange.

MDR strain	Ofloxacin				Gentamicin			
	0.35%	0.55%	0.75%	0.95%	0.35%	0.55%	0.75%	0.95%
PA_1	78.5	0.0	0.0	0.0	0.0	50.0	50.0	0.0
PA_2	80.0	160.0	0.0	0.0	61.5	76.9	0.0	0.0
PA_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PA_4	50.0	0.0	0.0	0.0	58.3	83.3	0.0	0.0
PA_5	0.0	58.8	0.0	0.0	60.0	180.0	120.0	120.0
Mean±SE	52.1±18.6	54.7±37.6	0.0	0.0	45.0±15.0	97.6±28.3	42.5±12.1	30.0±16.1

orange. This was followed by mean loss of less than 50.0% to gentamicin after treatment with 0.75 µg/ml acridine orange. This was followed by mean losses of 45.0±15.0, 42.5±12.1 and 30.0±16.1% all to gentamicin after treatment with 0.35, 0.75 and 0.95 µg/ml, respectively.

The highest and lowest mean loss of RM therefore, were 97.6±28.3 and 30.0±16.1% after treatments with 0.55 and 0.95 µg/ml, respectively and both to gentamicin. There was no reduction in RM to ofloxacin after 0.75 and 0.95 µg/ml acridine orange treatments. In all, mean loss

of 50.0% or more of RM was recorded after treatment with 0.35 µg/ml (52.1±18.6%) and 0.55 µg/ml (97.6±28.3%) to gentamicin.

DISCUSSION

The antibiotics susceptibility profile of all five strains of *P. aeruginosa* before acridine orange treatment in this study showed that the five strains were sensitive to ofloxacin and gentamicin with mean±SE zones of inhibition of 14.6±9.5 and 8.4±4.4 mm, respectively. This means only 2 (25.0%) of the antibiotics recorded positive reactions when tested on the pathogenic organisms. By extension, this also implies that 6 (75.0%) of the antibiotics were completely resisted by the five *P. aeruginosa* strains. This is rather alarming as findings confirm the MDR nature of *P. aeruginosa* as reported by earlier authors (Radi and Rahman, 2010; Mukherjee et al., 2011). Findings in this study (in terms of antibiotic sensitivity profile) is disturbing because it ostensibly suggests that infections or diseases caused by MDR *P. aeruginosa* in the study environment can be treated successfully with only ofloxacin or gentamicin or a combination of both. This place the low income patients at a serious disadvantage as they may not be able to afford ofloxacin which is expensive and which showed almost twice sensitivity reaction compared with gentamicin.

Particularly worrisome, is the total resistance against augmentin, amoxicillin and cotrimoxazole because these drugs are used routinely to treat a myriad of human diseases. This same worry with particular reference to augmentin has been expressed by some authors (Oluremi et al., 2011). 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 variations from site to site and presence/absence of oxygen could affect the response of *P. aeruginosa* (a strict aerobe) to relevant antibiotics it is exposed to *in vitro*.

A pathogen is MDR when it is resistant to three or more antibiotics at any given time (Jan et al., 2002). Based on results obtained in this study, *P. aeruginosa* strains isolated from ear swab, urethral swab, wound swab and midstream urine were each resistant to augmentin, nalidixic acid, nitrofuratoin, cotrimoxazole, amoxicillin and tetracycline (6 drugs) representing 75.0% of the antibiotics tested. Only strain PA_3 (isolated from tracheal swab) resisted eight drugs representing 100.0% of antibiotics tested. This finding re-establishes the MDR nature of *P. aeruginosa* strains irrespective of the source or site from where they are isolated. The high prevalence of multiple antibiotic resistant *P. aeruginosa* strains in this study is a possible suggestion that very large population of *P. aeruginosa* organisms has been exposed to several antibiotics which is consistent with report of earlier studies(Oluremi et al., 2011).

Acridine orange dilutions of 0.35, 0.55, 0.75, and 0.95 µg/ml were used to treat and cure the five pathogenic strains of *P. aeruginosa* with the intent of reducing their RM significantly or eliminating them completely. The loss of 50 to 100% of RM after treatment with acridine orange subclinical dilutions of 0.35, 0.55, 0.75 and 0.95 µg/ml was used as the basis of establishing the curing effects of these dilutions. The use of 50% and above loss in RM 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 agents reflects the ability of such agents to inhibit plasmid replication at a concentration that does not affect the chromosome.

It has been reported that acridine orange and ethidium bromide are better curing agents for *P. aeruginosa* than sodium dodecyl sulphate (SDS) and elevated temperature (Kumar and Surendran, 2006). Earlier studies reported no effect of acridine orange on plasmids of *P. aeruginosa* (Pattnakik et al., 1995; Al-Amir et al., 1999). Reports of some other earlier studies stated a reversion in the resistance of some multiple drug resistant strains following exposure to acridine orange (Naomi, 1978; Darini, 1996; Adeleke and Odetola, 1997).

P. aeruginosa strain PA_1 (isolated from ear swab) recorded 78.5% loss of RM to ofloxacin after 0.35 µg/ml treatment with acridine orange. For the same strain, there was zero loss of RM to ofloxacin after 0.55, 0.75 and 0.95 µg/ml treatments with the agent. This somewhat implies that 0.35 µg/ml is a choice dilution of the agent which may enhance antibiotic sensitivity of this MDR *P. aeruginosa* strain of ofloxacin.

In case of strain PA_2 (isolated from urethral swab), RM losses of 80.0 and 160.0% to ofloxacin were recorded after 0.35 and 0.55 acridine orange treatments, respectively. Strain PA_4 (isolated from wound swab) recorded 50.0% loss of RM to ofloxacin after 0.35 µg/ml treatment with the curing agent. There was zero loss of RM to ofloxacin by strain PA_5 with 0.35 µg/ml treatment and 58.8% loss of RM to the same drug after 0.55 µg/ml acridine orange treatment. The mean±SE loss of RM of 52.1±18.6 and 54.7±37.6% to ofloxacin by all the MDR *P. aeruginosa* strains after 0.35 and 0.55 µg/ml acridine orange treatments, respectively, tends to suggest potency of either or both dilutions in the enhancement of sensitivity to ofloxacin in the course of treatment. In a related study, Otajevwo (2012) reported 0.35, 0.85 and 0.95 µg/ml dilutions of homodium (ethidium) bromide as significant enhancers of a multidrug uropathogenic *Escherichia coli* strain to some selected antibiotics.

Some authors have used dilutions of a non-antibiotic compound-thioridazine to reduce RM in some strains of *P. aeruginosa* (Mukherjee et al., 2011). Loss of RM to gentamicin by strains PA_1, PA_2, PA_4 and PA_5 after 0.35 µg/ml acridine orange treatment was recorded as 0.0, 61.5, 58.3 and 60.0%, respectively with a less than 50.0% mean±SE dilution (that is 45.0±15.0%). After 0.55 µg/ml treatment with acridine orange, PA_1, PA_2, PA_4 and PA_5

strains showed loss of RM of 50.0, 76.9, 83.3 and 180.0%, respectively with a mean±SE percentage loss of 97.6±28.3. Acridine orange dilutions of 0.75 and 0.95 µg/ml recorded less than 45.0 and 35.0% loss of RM, respectively. These findings also suggest 0.55 µg/ml dilution and to a lesser extent, 0.35 µg/ml as potential enhancers of antibiotic sensitivity in MDR *P. aeruginosa* pathogens.

Sensitivity enhancement effect of the subclinical acridine orange dilutions on the minimum inhibitory concentration (MIC) of gentamicin as it affected MDR *P. aeruginosa* strain (isolated from midstream urine) showed a two-fold (5.0 µg) and four-fold (2.5 µg) reductions in MIC of gentamicin as recorded for 0.35 and 0.55 µg/ml acridine orange dilutions, respectively. Some authors had reported similar findings on MDR *Staphylococcus aureus* strains (Otajevwo and Momoh, 2013). 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 health care providers as well as reduce development of resistance (NCCLS, 2000; McGowan and Wise, 2001).

In this study, the MIC of gentamicin (an aminoglycoside) which is 10 µg (based on long standing research) was reduced to 5 µg (two fold reduction) and 2.5 µg (four fold reduction) by acridine orange subclinical dilutions of 0.35 and 0.55 µg/ml, respectively as tested on a multiple resistant drug strain of *P. aeruginosa* isolated from the urinary tract of a patient. A similar report had been made by an author which stated that 0.35, 0.45, 0.75, 0.85 and 0.95 µg/ml dilutions of homodium bromide reduced the MIC of gentamicin to 2.5, 5, 5, 2.5 and 2.5 µg, respectively when tested on an MDR strain of uropathogenic *E. coli* (Otajevwo, 2012). The implication of findings in this study is that when doses of either 0.35 or 0.55 µg/ml or both are incorporated into the manufacture of gentamicin or any other related aminoglycoside, and then administered to a patient diagnosed to be suffering from a disease caused by any MDR *P. aeruginosa* 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 *in vivo*. In a related work, Kohler (2010) showed that the resistance of *P. aeruginosa* to tetracycline efflux was reduced from MIC 0.032 to 0.004 µg/ml (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 tuberculosis*. 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).

Conclusion

All five MDR *P. aeruginosa* strains used in this study showed 100.0% resistance against augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. Fortunately, all five MDR (but one, PA_3) strains showed sensitivity to ofloxacin and gentamicin. This finding implies that augmentin and amoxicillin for example, which are used routinely to treat a gamut of human diseases may not yield good result in terms of curing human diseases due to MDR *P. aeruginosa* and therefore, their routine prescription should be discouraged. Sensitivity to ofloxacin and gentamicin on the contrary, suggests that either of the two drugs or a combination of both may proof successful in tackling human diseases due to MDR *P. aeruginosa.* Four of the five strains were each resistant to 6 (six) of the antibiotics tested and this qualifies them as multiple drug resistant organisms. This finding re-establishes the MDR nature of *P. aeruginosa* strains irrespective of source or site they are isolated from. The high prevalence of multiple antibiotic resistant *P. aeruginosa* strains in this study therefore is a possible suggestion that very large population of *P. aeruginosa* organisms has been exposed to several antibiotics (that is, drug abuse). Symptomatic and asymptomatic patients are advised therefore to desist from self medication for any justifiable reason. They should ensure drugs are taken based on prescription by a qualified doctor after proper evaluation.

Acridine orange of 0.35 and 0.55 µg/ml recorded loss of RM of 52.1±18.6 and 54.7±37.6% to ofloxacin, respectively and then loss of RM of 45.0±15.0 and 97.6±28.3% to gentamicin, respectively. This is suggestive of the potency of either or both dilutions in the enhancement of sensitivity to ofloxacin and gentamicin in the course of therapy *in vivo* as they may be able to eliminate drug resistance plasmids.

Finally, acridine orange dilutions of 0.35 and 0.55 µg/ml recorded two- and four-fold reductions in MIC of gentamicin, respectively when tested on a MDR strain of *P. aeruginosa.* Again, the aforementioned dilution if incooperated into drug regimens may produce better effect and results in terms of cure of a disease which will require four times the concentration of the new MICs to function *in vivo*. A decrease in the MIC of an antibiotic will make the particular antibiotic more effective in treatment of a *Pseudomonas* disease.

SUGGESTIONS FOR FURTHER STUDIES

The determination of resistance loss by MDR *P. aeruginosa* strains after treatments with 0.35, 0.55, 0.75 and 0.95 µg/ml acridine orange dilutions may not be totally convincing. It is recommended therefore, that the effects of these dilutions in the 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.

REFERENCES

Abdi-Ali AA, Rahmani-Badi T, Falsafi N, Nikname V (2007). Study of antibiotics resistance by efflux in clinical isolates of *Pseudomonas aeruginosa*. Pak. J. Biol. Sci. 10:924-927.

Aendekerk S, Diggle SP, Song Z, Hoiby N, Williams P, Camara M (2005). The MexGHI-OpmD multidrug efflux pump controls growth, antibiotic susceptibility and virulence in *Pseudomonas aeruginosa* via 4- quinolone dependent cell-to-cell communication. Microbiology 151:1113-1125.

Adeleke OE, Odelola HA (1997). Plasmid profiles of multiple drug resistant local strains of *Staphylococcus aureus* in two child care centers. J. infect. Dis. 178:577-580.

Akortha EE, Aluyi HAS, Enerijiofi KE (2011). Transfer of amoxicillin resistance gene among bacteria isolates from sputum of pneumonia patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. J. Med. Med. Sci. 2(7):1003-1009.

Bauer AW, Kirby WMM, Sherris JC, Turk M (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45:493-496.

Bonomo RA, Szabo D (2006). Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. Clin. Infect. Dis. 43:49-56.

Byron F, Brehm S, Eric AJ (2003). Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by these sesquiterpenoids. Antimicrob. Agents Chemother. 47(10):3357-3360.

Cowan ST, Steel KJ (1993). Manual for the identification of medical bacteria 3rd ed. Cambridge University Press, London, New York, Rockville, Melbourne and Sydney. 150p.

Defez C, Fabbro-peray P, Bouziges N, Gouby A, Mahamat A, Daures JP, Sotto (2004). Risk factors for multidrug-resistant *Pseudomonas aeruginosa* nosocomial infection. J. Hosp. Infect. 57: 209-216.

Crowle AJ, Douvas SG, May MH (1992). Chlorpromazine: a drug potentially useful for treating Mycobacterial infections: *Chemotherapy* 38:410-419.

Darini AL (1996). A genetic study of a *Staphylococcus aureus* plasmid involving cure and transference. Rev. Paulista Med. 114(1):1068-1072.

Lomovskaya O, Warren MS, Lee A, Galazza J, Fronko R, Lee M, Blais J, Cho D, Chamberland S, Renau T, Leger R, Hesker S, Hoshino K, Ishido H, Lee VJ (2001). Identification and characterization of inhibitors of multidrug Resistance Efflux Pumps in *Pseudomonas aeruginosa*: Novel agents for combination Therapy. J. Antimicrob. Chemoth. 45(1):105-116.

Govan JRW, Deretic V (1996). Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Microbiol. Rev. 60:539-574

Jan MB, John DT, Sentry P (2002). High prevalence of oxacillin resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: Results from SENTRY antimicrobial surveillance program, 1998-1999. Antimicrob. Agent Chemother. 46:879-881.

Kohler NO (2010). Non-antibiotics Reverse Resistance of Bacteria to Antibiotics *in vivo.* J. Antimicrob. Chemother. 24(5):751-754.

Kumar R, Surendran PK (2006). Plasmid mediated antibiotic resistance in *Pseudomonas cepacia* from brackish water aquaculture farms. Indian J. Fish 53(3): 263-269.

Manikandan S, Ganesapandian S, Singh M, Kumaraguru AA (2011) Emerging of multidrug resistance human pathogens from urinary tract infections. Curr. Res. Bacteriol. 4:9-15.

Mansouri S, Norouzi F, Moradi M, Nakhaee N (2011). Comparison of virulence factors among clinical isolates of *Pseudomonas aeruginosa* producing and non-producing extended spectrum beta-lactamases. Curr. Res. Bacteriol. 4:85-93.

McGowan AP, Wise R (2001). Establishing MIC breakpoints and the interpretation of *in vitro* susceptibility tests. J. Antimicrob. Chemother. 48:17-28.

Mukherjee S, Chaki S, Das S, Sen S, Datta SK, Dastidar SG (2011). Distinct synergistic action of piperacillin and methylglyoxal against *Pseudomonas aeruginosa.* Indian J. Exp. Biol. 49:447-551.

Naomi D (1978). Plasmid determined drug resistance. In: Reeves DS, Phillip I, Williams JD, Wise R (eds.), Laboratory Methods in anti-microbial chemotherapy. Edinburgh, Churchill: Livingstone. pp 56-63.

NCCLS (2000). Methods for Dilution, Antimicrobial Susceptibility Tests for Bacteria that grow aerobically. Approved Standard (5th edn). Wayne, PA, USA.

Neu HC (1993). Overview of mechanisms of bacterial resistance. Diagn. Microbiol. Infect. Dis. 12:109-116.

Ochei J, Kolhatkar A (2008). Medical Laboratory: Theory and Practice, 10th edn. New Delhi: Tata McGraw-Hill Publishing Company. 1338p.

Otajevwo FD (2012). Sensitivity Enhancement of Multidrug Resistant Urinary Tract *Escherichia coli* Isolate to Some Commonly Used Antibiotics after Treatment with Non-Toxic Laboratory Concentrations of Homodium Bromide. IOSR J. Pharm. 2(3):540-568.

Otajevwo FD, Momoh SA (2013). Resistance marker loss of multi-drug resistant (MDR) *Staphylococcus aureus* strains after treatment with dilutions of acridine orange. J. Appl. Med. Sci. 2(2):43-62. ISSN: 2241-2328

Oluremi BB, Idowu AO, Olaniyi JF (2011).Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a Teaching Hospital in Southwestern Nigeria. Afr. J. Microbiol. Res. 5(22):3658-3663.

Ranjbar R, Owlia P, Saderi H, Bameri Z, Izadi M, Jonaidi N (2007). Isolation of clinical strains of *Pseudomonas aeruginosa* harboring different plasmids. Pak. J. Biol. Sci. 10:3020-3022.

Radi F, Rahma H (2010). Study the Effect of Ethidium bromide SDS and Elevated Temperature on Stability of Multiple Antibiotic Resistances plasmid of *Pseudomonas aeruginosa*. Iraqi J. Biotechnol. 9(4):796-811.

Ramesh A, Halami, PM Chandrasekhar A (2000). Ascorbic acid induced loss of a pediocin-encoding plasmid in *Pediococcus acidilactici* CFR K7. World J. Microbiol. Biol. 16:695-697.

Salisbury V, Hedges RW, Datta N (1972). Two modes of curing transmissible plasmids. J. Gen. Microbiol. 70:443-452.

Shahid MA, Malik S, Sheeba (2003). Multidrug resistant *Pseudomonas aeruginosa* strains harboring plasmids and AmpC L-lactamases isolated from hospitalized burn patients in a tertiary care hospital of North India. FEMS Microbiol. Lett. 228:181-186.

Shiram V, Jahagirdar S, Latha C, Kumar V, Puranik V, Rojatkar S (2008). A potential plasmid curing agent, 8- epidiosbulbin E acetate from Dioscoreabulbifera L against multidrug resistant bacteria. Int. J. Antimicrob. Agents 32(5):405-410.

Sonstein SA, Baldwin JN (1972). Loss of the penicillinase plasmid after treatment of *Staphylococcus aureus* with sodium dodecyl sulphate. J. Bacteriol. 109:262-265.

Stainer RY, Adelberg EA, Ingraham JL (1984). General Microbiology, 4th edn. The Macmillan Press Ltd, Basingstoke London.

Wurmb-Schwark N, Cavelier L Cortopassi GA (2006). A low dose of ethidium bromide leads to an increase of total mitochondrial DNA while higher concentrations induce DNA 4997 deletion in a human neuronal cell line. Mutat. Res. 596(2):57-63.