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EFFECT OF DIFFERENT CONCENTRATIONS OF ACRIDINE ORANGE ON SELECTED GRAM POSITIVE AND GRAM-NEGATIVE ORGANISMS

***[1]Inyang, N. J., [1]Babatope I.O., [2]Adewuyi G.M., [3]Ikheloa J., [2]Olagboye J.A., [4]Umoh N.O., [1]Omokhodion O. M.**

[1]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma Edo State, Nigeria [2]Medical Microbiology Department, Irrua Specialist Teaching Hospital Irrua, Edo State Nigeria. [3]Department Obstetrics and Gynecology, Irrua Specialist Teaching Hospital Irrua, Edo State Nigeria. [4]Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria
*Corresponding Author's Email: nyohoinyang@yahoo.com

ABSTRACT

This investigation determined the effect of different concentrations of Acridine Orange (AO) on selected Gram positive and Gram negative organisms. Twenty (20) clinical isolates of multidrug-resistant strains that included *Staphylococcus aureus* (5;25%), *Escherichia coli* (6;30%) *Pseudomonas aeruginosa* (5;25%) and *Klebsiella species* (4;20%), were obtained from the Department of Medical Microbiology, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria. The differences in drug susceptibility profiles of the isolates before and after AO-curing, were evaluated and compared. In comparison, *Staphylococcus aureus* strains 1, 2 & 4; *E.coli* strain 1; *P. aeruginosa* strain 1&4, and *Klebsiella* species strains 1&3 showed statistically significant differences ($P<0.05$) in curing rates unlike *Staphylococcus aureus* strain 3 and *E. coli* strains 5&6 ($P>0.05$). *E. coli* strains 2&5, *P. aeruginosa* strains 2&3, and *Klebsiella* species strain 4, showed sensitivity to a minimum of one antibiotic and a maximum of two to the three concentrations of AO used, while *Staphylococcus aureus* strain 5, *E.coli* strain 3 and *Klebsiella* species strain 2, showed complete resistance to all the antibiotics used. Overall, curing was achieved in 25%, 35% and 75% of the 50ug/ml, 75ug/ml and 100ug/ml AO-treated isolates respectively; depicting the concentration dependent curing potential of Acridine Orange.

Key words: Acridine orange, curing, Bacterial Plasmid, Microbial organisims

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INTRODUCTION

Antibiotics resistant infections have become more challenging to treat with existing antibiotics leading to more complex infections (Carlet *et al*., 2011; Finley *et al*., 2013). Antimicrobial resistance is a well-known clinical and public health problem (Oteo *et al*., 2002; Okonko *et al*., 2009a). As resistance develops to first line antibiotics, therapy with new broader spectrum antibiotics that are often more expensive is used, and likelihood for development of resistance to the new class of drugs (CLSI, 2006; Radha *et al*., 2012). Drug resistance is more frequently encountered in hospital-acquired pathogens, but in recent years, the incidence of antibiotic resistant pathogens in community-acquired infections has also been on the rise (Hooton and Levy, 2001; Radha *et al*.,2012).

Organisms isolated from clinical samples like *P. aeruginosa* and *Enterobacter* species are resistant to most chemotherapeutic agents (Okonko et *al*., 2009a), while pathogenic isolates of *E.coli* have relatively large potentials for developing resistance (Sahm *et al*., 2001; Karlowsky *et* al., 2004; Okonko *et al*., 2009a).

Microbial resistance to drugs and other antimicrobial agents has been attributed to a variety of factors, which has to do with the intrinsic ability of microbes to develop resistance adaptively or acquired from the environment. This ability may be due to mutation at the chromosomal level and/or the presence of the plasmid in the genome of the microbes.

Both chromosomal based resistance and plasmid-borne resistance are often associated with the transfer of gene or plasmids coding for such resistance. The transfer may be through integrons and transposons or may sometimes involve transduction with the help of lysogenic phages such as T_4 phage or any of the other horizontal gene

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transfer methods such as conjugation and transformation or through vertical gene transfer. Plasmids are an extra-chromosomal unit in bacteria capable of independent replication within its host. The presence of plasmids usually confer specialised properties on microbes as a plus to the microbes' intrinsic ability. The presence of plasmids as an extra-chromosomal unit in most bacteria have been identified as one of the tools through which they resist drugs and other antimicrobial agents. The most common and important mechanism through which *Staphylococcus aureus* can become resistant against β-lactams is by expressing β-lactamases, for example, extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC enzymes, and carbapenem-hydrolyzing β-lactamases (Paterson and Bonomo, 2005, Poirel *et al*., 2007, Jacoby, 2009).

Drug resistance is an alarming problem worldwide and is spreading rapidly due to overuse, self-medication and non-therapeutic use of antimicrobials (Slama *et al*., 2015i). Antimicrobials themselves act as a selective pressure that allows the growth of resistant bacteria within a population and inhibits susceptible bacteria (levy, 1994). Drug resistance property in bacteria is usually born in R-plasmids, which can be disseminated to diverse population and regions causing worldwide problems. R-plasmid from resistant strains of an organism may transfer to a sensitive counterpart which can show the same drug resistance in the donor train (Brussow *et al*., 2004).

Plasmid-mediated multidrug resistance (MDR) presents serious challenges in the treatment of infectious diseases (Pratibha *et al.*, 2015). The use of plasmid curing agent in association with antibiotics may serve as a feasible way to control the development and spread of antibiotic resistance encoded by antibiotic resistance plasmids (R-plasmids) (Molnar *et al*., 2003, Gunics *et al.*, 2000, Pratibha *et al.*, 2015). However, the majority of the known conventional plasmid curing agents such as acridine orange, ethidium bromide, sodium dodecyl sulphate are toxic or mutagenic and hence not recommended for in vivo testing and therapeutic applications (Amábile Cuevas *et al., 2004*, Pratibha *et al.*, 2015).

Acquired resistance is said to occur when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. This can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes or from a combination of these two mechanisms. Unlike intrinsic resistance, traits associated with acquired resistance are found only in some strains or subpopulations of each particular bacterial species.

This study was aimed at studying the effects of different concentrations of acridine orange on selected Gram positive and Gram negative organism in other to establish the best concentration for use. Intercalating dyes such as acriflavine, acridine orange, ethidium bromide and quinacrine have been successfully used in curing bacteria of plasmid. Acridine has also been successfully used by other researchers for plasmid curing in *E. coli* (Southhamer, *et al.,* 1963, Wechsler and Kline, 1980, Hahn and Ciak 1971).

MATERIALS AND METHODS

Sample Collection: Relying on an ethical approval, a total of 20 clinical isolates of multidrug-resistant bacteria were obtained from the Department of Medical Microbiology Laboratory at Irrua Specialist Teaching Hospital, Irrua, Edo-state of Nigeria. The isolates included *Staphylococcus aureus* (5), *Escherichia coli* (6), *Pseudomonas aeruginosa* (5), *and Klebsiella spp* (4). These isolates were inoculated aseptically into sterile nutrient agar slants, which were properly labelled and stored at 8^0C in the refrigerator.

Antibiotic sensitity test: The isolated organisms were checked for antibiotic resistance with ceftazidime (30µg) cefuroxime (30µg), gentamicin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), amoxicillin/clavulanate (30µg) and ampicillin (10µg). The Kirby-Bauer standardised disc diffusion method (Bauer *et al*., 1996), was employed for antibiotic susceptibility of the isolates. Each isolate was inoculated into peptone water broth and the inoculums were used to seed nutrient agar plate. The antibiotic disc was aseptically placed on the seeded

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plates. This was incubated at 37^0c for 24 hours. The antibiotic diffuses into the surrounding medium and establishes a gradient of concentration around the disc. The concentration of the drugs was highest in the agar near the disc. As the distance from the disc increases there was a reduction in the drug concentration (Baker *et al.*, 2001; NCCLS, 2002; Cole, 2005; Chessbrough, 2006; Okonko *et al*; 2009 a,b). 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).

The results were expressed as 'susceptible (S)' or 'resistant ®' according to the criteria developed by national committee for clinical laboratory standards (NCCLS 2002) and Manual of antimicrobial susceptibility testing guidelines (Cole 2005; Chessbrough, 2006; Okonko *et al*; 2009 a,b). Multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested (Oteo *et al.*, 2005).

Plasmid curing by acridine orange dye: In order to determine the location (plasmid-borne or chromosomal) of the drug resistance markers to curing (elimination), experiments were performed by acridine orange dye (Salisbury *et al.*, 1972). Curing of the plasmid in the bacteria isolates was performed by the method of Tomoeda *et al.* (1968) with slight modification. Acridine orange (50µg/ml, 75µg/ml and 100µg/ml) were used to cure the plasmid.

An overnight culture of each organism in brain heart infusion broth was done. 1ml of each dilutions/ concentrations of acridine orange was added to 20ml of brain heart infusion broth, to obtain the desired concentrations.

The broth mixture was inoculated with (50µl) of each isolate collected from an overnight culture grown in brain heart infusion broth. It was properly mixed. Positive and negative controls were also run. Positive control contained only cells and no acridine orange while negative control contained only acridine orange and no cells, and incubated in water bath at 37^0c for 24hours with minimum agitation at intervals. It was then subcultured into the nutrient agar and was incubated at 37^0c for 24hours. Antibiotic sensitivity was done on the cured cells (as described above).

The cured plasmid cells were detected by comparing the development of bacterial colonies on the cured plates before and after curing. Cured markers were determined by comparison between the pre and post curing antibiograms of isolates. Loss of resistance markers gives an indication that the markers were probably located on a plasmid and not the chromosomes.

RESULTS.

This study looked at 1 Gram-positive and 3 gram negative organisms commonly encountered in a clinical setting to demonstrate their resistant profile. The result showed that from the five strains of *Staphylococcus aureus* that represented the Gram-positive organisms tested with 50ug/ml, 75ug/ml, and 100ug/ml of AO, strains1, 2 and 4 showed significant differences in the curing rate ($p<0.05$), while strain 2 showed no significant difference ($p>0.05$). Strain 5 however, remained the same before and after curing.

The results showed also that both 50ug/ml and 75ug/ml of AO, had effect in 2 out of the 5 strains of *Staphylococcus aureus* used, while 100ug/ml of AO had an effect on 3 strains out of the 5 strains used (*see* Table 1). Regarding the effect of the different concentration of AO on the 6 strains of *E. coli* used, it was observed that 50ug and 75ug/ml of AO had effect on strains 1 and 2 respectively, while the 100ug/ml of AO had effect on 4 strains (*see* Table 2).

The results of the 5 strains of *P. aeruginosa* tested revealed that both 50ug and 75ug/ml of AO had an effect on only 1 strain, while 100ug/ml of AO had an effect on 5 of the strains (*see* Table 3). Table 4 shows the profile of *Klebsiella* strains tested The 50ug/ml and 75ug/ml of AO had effect on 1 and 2 strains respectively, while 100ug/ml of AO was effective on 4 strains. Overall, 50ug/ml, 75ug/ml and 100ug/ml of AO cured 25%, 35% and 75% of the isolates respectively.

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TABLE 1: SENSITIVITY PROFILE OF STAPHYLOCOCCUS AUREUS BEFORE AND AFTER TREATMENT WITH 50 µg/ml, 75 µg/ml, AND 100 µg/ml. CONCENTRATIONS OF ACRIDINE ORANGE

ISOLATES	TEST (Curing)	CONC. OF AO	zones of inhibition(mm) around the antibiotic disc CAZ	CRX	GEN	CPR	OFL	AUG	AMP	X^2	P-value	REMARK
Staph. aureus	Before		23	0	0	0	0	0	0			
Strain 1	After	50µg/ml	18	0	0	0	0	17	0			
		75µg/ml	23	0	0	0	0	17	0	96.65	P=0	P<(0.05)
		100µg/ml	23	0	15	20	16	18	0			
Staph.aureus	Before		0	0	18	12	0	26	0			
Strain 2	After	50µg/ml	0	0	18	15	14	0	0			
		75µg/ml	0	0	18	16	15	0	0	87.49	P=0	P< (0.05)
		100µg/ml	0	0	18	17	16	0	0			
Staph.aureus	Before		0	0	0	25	24	0	0			
Strain 3	After	50µg/ml	0	0	0	11	19	0	0			
		75µg/ml	0	0	0	20	20	0	0	1.96	P=0.5	P>(0.05)
		100µg/ml	0	0	0	25	24	0	0			
Staph.aureus	Before		0	0	14	15	0	0	0			
Strain 4	After	50µg/ml	0	0	15	10	0	0	0			
		75µg/ml	0	0	16	12	0	0	0	70.13	P=0	P<(0.05)
		100µg/ml	19	15	18	17	16	0	0			
Staph. Aureus	Before		0	0	0	0	0	0	0			
Strain 5	After	50µg/ml	0	0	0	0	O	0	0			
		75µg/ml	0	0	0	0	0	0	0	NOT		
		100µg/ml	0	0	0	0	0	0	0	APPLIED		

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TABLE 2: SENSITIVITY PROFILE OF ESCHERICHIA COLI BEFORE AND AFTER TREATMENT WITH 50 µg/ml, 75 µg/ml, AND 100 µg/ml. CONCENTRATIONS OF ACRIDINE ORANGE

ISOLATES	TEST (Curing)	Zones of inhibition(mm) around the antibiotic disc CAZ	CRX	GEN	CPR	OFL	AUG	AMP	X^2	P-value	REMARK
E. coli	Before	21	11	17	0	0	0	0			
Strain 1	50µg/ml	17	12	15	0	0	0	0			
	75µg/ml	20	13	19	0	0	0	0	86.65	P=0	P<(0.05)
	100µg/ml	18	11	17	0	0	0	0			
E.coli	Before	0	0	0	0	0	0	0			
Strain 2	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	0	0	0	15	0	0	0			
E.coli	Before	0	0	0	0	0	0	0			
Strain 3	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	0	0	0	0	0	0	0			
E.coli	Before	0	0	0	0	0	0	0			
Strain 4	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	0	18	0	0	0	0	0			
E.coli	Before	0	0	0	0	0	0	0			
Strain 5	50µg/ml	0	0	0	0	O	0	0			
	75µg/ml	0	0	0	21	13	0	0	0.9	p= 0.9	P>(0.05)
	100µg/ml	0	0	0	22	17	0	0			
E.coli	Before	0	0	0	0	0	0	0	32.34	P=0.19	P> (0.05)
Strain 6	50µg/ml	18	0	0	0	0	0	0			
	75µg/ml	18	0	0	0	0	0	0			
	100µg/m	20	16	13	0	0	0	0			

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TABLE 3: SENSITIVITY PROFILE OF PSEUDOMONAS AERUGINOSA BEFORE AND AFTER TREATMENT WITH 50 µg/ml, 75 µg/ml, AND 100 µg/ml. CONCENTRATIONS OF ACRIDINE ORANGE

ISOLATES	TEST (Curing)	Zones of inhibition(mm) around the antibiotic disc CAZ	CRX	GEN	CPR	OFL	AUG	AMP	X^2	P-value	
P. aeruginosa	Before	0	0	13	22	15	0	0			
Strain 1	50µg/ml	18	16	16	21	17	0	0			
	75µg/ml	16	0	13	22	15	0	0	89.3	P=0	P<(0.05)
	100µg/ml	17	0	12	23	15	0	0			
P. aeruginosa	Before	0	0	0	0	0	0	0			
Strain2	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	14	0	0	0	0	0	0			
P. aeruginosa	Before	0	0	0	0	0	0	0			
Strain 3	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	0	0	16	20	15	0	0			
P. aeruginosa	Before	0	0	14	15	0	0	0			
Strain 4	50µg/ml	0	0	15	10	0	0	0			
	75µg/ml	0	0	16	12	0	0	0	80.5	P=0	P<(0.05)
	100µg/ml	19	15	12	17	16	0	0			
P. aeruginosa	Before	0	0	0	0	0	0	0			
Strain 5	50µg/ml	0	0	0	0	O	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	18	0	0	0	0	0	0			

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TABLE 4: SENSITIVITY PROFILE OF KLEBSIELLA SPECIES BEFORE AND AFTER TREATMENT WITH 50 µg/ml, 75 µg/ml, AND 100 µg/ml CONCENTRATIONS OF ACRIDINE ORANGE

ISOLATES	TEST (Curing)	Zones of inhibition(mm) around the antibiotic disc									
		CAZ	CRX	GEN	CPR	OFL	AUG	AMP	X^2	P-value	REMARK
Klebsiella 1	Before	0	0	0	26	24	0	0			
	50µg/ml	0	0	0	20	15	0	0			
	75µg/ml	0	0	0	19	17	0	0	32.9	P=0	P<(0.05)
	100µg/ml	13	0	0	21	18	0	0			
Klebsiella 2	Before	0	0	0	0	0	0	0			
	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	0	0	0	0			
	100µg/ml	0	0	0	0	0	0	0			
Klebsiella 3	Before	0	0	24	0	0	0	0			
	50µg/ml	15	11	19	0	0	0	0			
	75µg/ml	18	20	19	0	0	0	0	36.2	P=0	P<(0.05)
	100µg/ml	22	26	27	0	0	0	0			
Klebsiella 4	Before	0	0	0	0	0	0	0			
	50µg/ml	0	0	0	0	0	0	0			
	75µg/ml	0	0	0	15	0	0	0			
	100µg/ml	0	0	0	18	0	0	0			

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DISCUSSION

Plasmid curing is the treatment of bacteria cells that interferes with plasmid replication. This treatment leads to the elimination of plasmid from cell culture using plasmid curing agents (chemical or physical agents), which would result in the production of plasmid-less strains from the cell culture. Bacteria with stable plasmids can be treated with curing agents. These include chemical and physical agents, some of which can mutate DNA, interfere specifically with its replication or affect particular structural component or enzymes of the bacterial cells. Laboratory methods are therefore needed to detect acquired resistance in bacterial species that are not intrinsically resistant. These same methods are used for monitoring rates of acquired resistance as a means of combating the emergence and spread of acquired resistance traits in pathogenic and non-pathogenic bacteria species. Acquired resistance results from successful gene change and/or exchange that may involve: mutation or horizontal gene transfer via transformation, transduction or conjugation. (Martinez and Baquero, 2000; Kolář *et al*., 2001; Kumar and Schweizer, 2005; Higgins,2007).

In this study, we evaluated the sensitivity of the isolates to the antibiotics commonly used in our study environment and found that the most (entire) isolates showed multiple drugs resistance before curing with AO, in order to ascertain if this multiple resistance was inert or acquired via a plasmid. The antibiotic susceptibility/sensitivity pattern of all isolates before treatment with Acridine Orange showed that all isolates were completely resistant to ampicillin (100%) and almost completely resistant to amoxicillin-clavulanate (95%) and cefuroxime (95%). They also showed high resistance to ceftazidime (90%) ofloxacin (85%), gentamicin (70%) and ciprofloxacin (70%).

The total resistance recorded against ampicillin (100%) and amoxicillin/clavulanate (95%) is worrisome because these drugs are used routinely to treat a myriad of human diseases in our setting. A similar case was observed by Omogberale *et al*., 2014 were 100% resistance for amoxicillin and ampicillin was recorded in their study on antibiotic resistance pattern of Staphylococcus aureus isolated from the high vaginal swab and urethral swab. The same worry with particular reference to amoxicillin was expressed by Otajevwo and Mommoh (2013) were 100% resistance to amoxicillin was recorded on their report on resistance marker loss of multidrug-resistant Staphylococcus aureus strains.

The high amoxicillin resistance rate of 95% in this study is comparable to those of other researchers were 92% resistance was reported by Akortha and Egbule (2008) on their study on the transfer of tetracycline resistance gene between replicons in some enteric bacteria of diarrhoeal origin from some settings (hospital) in south-south Nigeria.

The rate of isolates resistance to gentamicin (70%) in this study was high. This does not agree with the report (17.9%) of Yah *et al*., 2008, on their study on the plasmid-borne antibiotic marker of Serratia marcescent and increased prevalence in HIV & AIDs patient and the reports (17.7%) of Akortha and Filgona (2009). on their study on the transfer of gentamicin resistance genes among Enterobacteriaceae isolated from an outpatient with urinary infection attending 3 hospitals in Mubi Adamawa state.

The results of this study showed high-level resistance of all isolates towards cefuroxime (95%) this agrees with the findings of Tula and Iyoha(2014) who reported high resistance towards cefuroxime(93.1%) on their study on distribution and antibiotic susceptibility pattern of bacterial pathogens causing urinary tract infection in Mubi General Hospital Yola-Nigeria. Similarly, Adeleke *et al*., 2014 reported high resistance towards cefuroxime (81.8%) on their study on the susceptibility of some gram positive and gram negative bacteria against some brand of amoxicillin. However, this is contrary to the report of Yah and Eghafona (2008) who reported low resistance (6.6%) of their diarrhoea isolates toward cefuroxime on their study on Plasmid: A vehicle for rapid transfer of antibiotic resistance markers of Salmonella species in animals.

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Elimination of plasmid with dye inhibit replication and results in plasmid-free segregants during subsequent cell division (Akortha and Aluyi., 2002). Curing of the resistant plasmid with dye gives an indication that the mechanism of resistance is plasmid mediated.

In this study, after treatment with acridine orange dye at 50µg/ml, 70µg/ml and 100µg/ml concentrations isolate that showed resistance to some antibiotics became sensitive to it. Improvement of zone of inhibition of isolates that were first susceptible to some antibiotics was observed a similar observation was reported by Otajavwo and Momoh (2013) on their study on Resistance marker loss of multi-drug resistant(MDR) Staphylococcus aureus strain after treatment with dilutions of Acridine orange

Cured cells were achieved in many isolates at the various concentrations used, where the comparison of the difference in curing rate for *Staphylococcus aureus* 1, 2, and 4: E. coli 1: *P.aeruginosa* 1 and 4 and *Klebsiella* species 1 and 3. Were statistically significant. $P< 0.05$. But *Staphylococcus aureus* 3, *E. coli* 5 and 6 was not statistically significant. $P> 0.05$.

E.coli 2 showed sensitivity only to ciprofloxacin at 100 µg/ml, E. coli 4 showed sensitivity only to cefuroxime at 100 µg/ml, while *E. coli* 5 showed sensitivity to ciprofloxacin and ofloxacin both at 75 µg/ml and 100 µg/ml concentrations of acridine orange treatment while they were completely resistant to all other antibiotic used before and after curing. A similar case was observed for *P. aeruginosa* 2 which was only sensitive to ceftazidime at 100 µg/ml and *P. aeruginosa* 3 which was only sensitive to gentamicin ciprofloxacin and ofloxacin at 100 µg/ml we also noticed that *Klebsiella* pneumonia 4 was only sensitive to ciprofloxacin at 100 µg/ml. statistical analysis was not possible because of few data. This could be as a result of mutation of other antibiotic genes in the isolates, with no mutation of these particular genes to which the isolates are sensitive to. This can be linked to the fact that Acridine orange dye (mutagenic) are said to cause potential mutation on the host chromosome which interferes with the functional analysis of the plasmid (Segen's Medical Dictionary, 2012). Also, the isolates coming from a hospital setting may have an impact on the sensitivity pattern.

Staphylococcus aureus 5, *E. coli* 3 and *Klebsiella* species 2 showed complete resistance to all antibiotics used before and after curing with acridine orange dye treatment. And thus statistical analysis was not applicable as there were no comparisons to be made. This shows that the encoding genes of resistance to antibiotics possessed by these isolates are located in the chromosomal DNA. It could be that these isolates are hospital acquired (nosocomial) since antibiotics are frequently used within the hospitals as such these types of bacteria and their resistance to antibiotics are different from bacteria outside of the hospital settings as they are more difficult and serious to treat. (Brusaferro *et al*., 2015).

When the isolates were treated with 75µg/ml concentration of acridine orange 5% loss of their amoxicillin/clavulanate resistance was observed in this study, Both using 75 µg/ml concentrations of acridine orange treatment. In this study, 95% resistance to amoxicillin/clavulanate for *Pseudomonas aeruginosa*, *Klebsiella* species, and *E. coli* was observed this is comparable to the high resistance reported from the study of Aluyi *et al*., 2013 using 10% SDS with a curing rate of 71.4% resistance to amoxicillin for *Pseudomonas aeruginosa*, 86.9% resistance for *Klebsiella* species and 71.4% resistance for *E.coli* were reported on their study on Multiple antibiotic resistance among bacteria isolated from hospital environment Benin-city Edo-state, Nigeria.

In this study, some isolates that were sensitive to a particular antibiotic before curing became resistant to the same antibiotic after curing with 50µg/ml, 75µg/ml and 100µg/ml concentrations of acridine orange treatment. Also, some isolates were susceptible at 50µg/ml concentration but became resistant at 75µg/ml and 100µg/ml concentrations. This could be as a result of the mutagenic ability of acridine orange dye. (Segen's Medical Dictionary, 2012), many isolates in this study retained their resistant ability to the same antibiotic they were resistant to after treatment with the three concentrations of acridine orange.this finding in this study is related to the findings of Elufisan and Adeleke, 2012 who reported that forty

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percent of their *Staphylococcus aureus* isolated from HVS Swab in their study survived after curing with mutagen (sodium dodecyl sulphate at a concentration of 250µg/ml and 500µg/ml). While eighty percent of the isolates from Wound swab survived after curing with mutagen on their study on the Epidemiological role of plasmid curing in the identification of *Staphylococcus aureus* route of infections.

It is, however, important to note that not all antibiotic resistance gene is plasmid mediated (Shoemaker *et al*., 1992). It may also be noted that copies of the plasmid lying closer to the membranes are completely eliminated by chemical agents while those lying close to the nucleus may escape the curing effect thereby one may observe partial curing (Jenks *et al*.,1995)

In this study, curing were achieved with 50µg/ml, 75 µg/ml and 100 µg/ml this correlates with the reports of Zaman *et* al., 2010 who reported that cured cells were achieved with a frequency rate of 5.55% (with 50 µg/ml) and 11.7% (with 75µg/ml). acridine orange treatment on their study on plasmid curing of Escherichia coli cells with Ethidium bromide, sodium dodecyl sulphate and acridine orange. The reports from Adeleke *et al*., 2014 also states that antibiotic resistance in the six bacterial isolates in their study after exposure to mutagens at 200µg/ml, 12 µg/ml and 10 µg/ml concentrations of SDS. Supports an R-plasmid mediated resistance from their study on the susceptibility of some Gram positive and Gram negative against some brands of amoxicillin-clavulanic acid.

The results obtained in this study shows that amongst the three concentrations of Acridine orange treatment used 100µg/ml were able to cure plasmid successfully at a higher rate than the other two concentrations (50µg/ml and 75µg/ml). These findings disagree with those of Zaman *et al*., 2010 who reported that no cured cells were obtained for 100 µg/ml concentration of acridine orange treatment with particular reference to Escherichia coli.

As it has been reported that most pathogens acquire their antibiotic resistance gene through transposons, chromosomes or another plasmid (Norma *et al.*, 2004) it is necessary to elaborate and enhance ways of controlling this phenomenon.

In conclusion, we found that any strain of the organism that showed an effect at 50ug and 75ug/ml also did at 100ug/ml of AO, but 100ug/ml of AO had effect where no other concentration had an effect. In summary, 50ug/ml of AO had an effect on 25 isolates, 75ug/ml of AO had an effect on 35% of the isolates and 100ug/ml of AO had an effect on 75% of the isolates. This result put 100ug/ml of AO as the best concentration for use when curing Gram positive and Gram negative bacteria.

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REFERENCES

Adeleke, O.E., Ogunniyi, O.A. and Olarinde, J.D. (2014). Susceptibility of some Gram-positive and Gram-negative bacteria against some brands of amoxicillin-clavulanic acid. *N Y Sci J.* 7(6):80-86.

Akortha, E.E. and Aluyi, H.S.A. (2002). Plasmid – mediated antibiotic resistance among some members of the *Enterobacteriaceae* from Diarrhoeal patients. *Trop. J. Environ. Sci. Health*. 5(1):13-16.

Akortha, E.E. and Filgona, J. (2009). Transfer of gentamicin resistance genes among *enterobacteriaeceae* isolated from the outpatients with urinary tract infections attending 3 hospitals in Mubi, Adamawa State. *Sci. Res. Essay* 4(8): 745 – 752.

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Akortha, E.E., and Egbule, O.S. (2008). Transfer of tetracycline resistance gene between replicons in some enteric bacteria of diarrhoeal origin from some hospitals in South-South Nigeria. *Afr. J. Biotechnol*. 7(18): 3178-3181.

AmábileCuevas C.F. and Heinemann J.A. (2004). Shooting the messenger of antibiotic resistance: plasmid elimination as a potential counter revolutionary tactic. *Drug Discov Today*; 9(11):465-467.

Baker, F.J., Silverton, R.E. and Pallister, C.J. (2001). Routine Bacteriological Examination Of Specimens in.: Baker And Silverton's Introduction To Medical Laboratory Technology. *Edward amold , London UK*, 299-315.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by a standard disc method. *Amer. J. Clin. Pathol*; 45: 494- 496.

Brusaferro S., Arnoldo L., Cattani G., Fabbro E., Cookson B., Gallagher R., Hartemann P., Holt J., Kalenic S., Popp W., Privitera G., Prikazsky V., Velasco C., Suetens C., Varela S. C.(2015) Harmonizing and supporting infection control training in Europe. *J Hosp Infect.*; 89(4):351-6.

Brüssow, H.H., Canchaya, C. and Hardt, W.D. (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *J.Microbiol.Mol.Biol.Rev*; 68:560-602.

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

Carlet, J., Collignon, P., Goldmann, D., Goossens, H., Gyssens, I.C. and Harbarth, S. (2011). Society's f failure to protect a precious resource: antibiotics. *Lancet* 378,369–371.

Chessbrough, M. (2006). District Laboratory Practice In Tropical; countries *cambrigde university press* 434.

Clinical and Laboratory Standards Institute, (CLSI) (2006). Performance standards for antimicrobial susceptibility testing, 16th informational supplement CLSI, NCCLS *Clinical and Laboratory Standards Institute,*100-116.

Cole, M.B. (2005). Manual of antimicrobial susceptibility testing. *American society for microbiology Press* 25-39.

Elufisan T.O. and Adeleke E.O (2012). Antibiotic susceptibility pattern of selectedstrains of *Staph aureus*(plasmid curing response of some selected strains of Staphylococcus aureus). Editor; agal, Publisher; Lambert Academic Publishing Germany.ISBN 978-3-659-15185-9.

Finley, R.L., Collignon, P., Larsson, J.D.G., McEwen, S.A., Li, X.Z. and Gaze, W.H. (2013). The scourge of antibiotic resistance: the important role of the environment. *Clin.Infect.Dis*; 57: 704–710.

Giguère, S. (2006). Antimicrobial Drug Action and Interaction in horses in. *Antimicrob Vet Med*; 1(4):449-462.

Gunics G, Motohashi N, Amaral L, Farkas S, Molnar J. (2000). Interaction between antibiotics and nonconventional antibiotics on different bacteria. *Int J Antimicrob Agents*; 14: 239–242.

Hahn, F.E. and Ciak, J. (1971). Elimination of bacterial episomes by DNA-complexing compound. *Ann. N.Y. Acad. Sci;* 182, 295-304.

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**). **Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

Higgins, C.F.(2007). Multiple Molecular Mechanisms for Multidrug Resistance Transporters. *Nature*; 446:749-757.

Hooton, T.M. and Levy. S.B. (2001). Antimicrobial resistance- a plan of action for community practice, *Ame. Fam. Physician*; 63(6): 1087-1098.

Jacoby G. A. (2009). AmpC β-lactamases. *Clin. Microbiol. Rev.* 22, 161–18210.

Jenks, P.J., Hu, Y.M., Danel, F., Mehtar, S. and Livermore, D.M. (1995). Plasmid-mediated production of class I (AmpC) β-lactamase by two Klebsiella pneumoniae. *J. Antimicrob. Chemotherp*. 35: 235-236.

Karlowsky, J.A., Jones, M.E., Draghi, D.C., Thornsberry, C., Sahm, D.F. and Volturo, G.A. (2004). Prevalence of antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in. *Ann. Clin. Microbiol. Antimicrob*; 3:7.

Kolàř, M. Urbànek, K. and Làtal, T. (2001). Antibiotic Selective Pressure and Development of Bacterial Resistance. *International Journal of Antimicrobial Agents*; 17:357-363.

Kumar, A. and Schweizer, H.P. (2005). Bacterial Resistance to Antibiotics: Active Efflux and Reduced Uptake. *Advanced Drug Delivery Reviews*; 57:1486-1513.

Levy, S.B. (1994). Balancing the drug-resistance equation. *Trends Microbiol*; 2(10): 341-342.

Martinez, J.L. and Baquero, F. (2000). Mutation frequencies and antibiotic resistance. *Antimicrobial Agents and Chemotherapy*. 44(7):1771-1777.

Molnar A, Amaral L, Molnar J.(2003). Antiplasmid effect of promethazine in mixed bacterial cultures. *Int J Antimicrob Agents*; 22:217–222.

National Committee For Clinical Laboratory Standards, (NCCLS) (2002). Performance standards for antimicrobial susceptibility testing twelfth informational supplement, *NCCLS document*; 100-112.

Norma, S.L., Anita, T., Dailia P.R., Eliane, M.F.R., Bianca R.Q., Ernesto, H. (2004). Antimicrobial resistance and R-plasmid in Salmonella spp. From swine and abatoir5 environment. *Pesq Vet Bres*. 24(2): 57-60.

Okonko, I.O., Soleye, F.A., Amusan, T.A., Ogun, A.A., Ogunnusi, T.A., and Ejembi, J. (2009a). Incidence Of Multi-Drug Resistance (MDR) Organisms In Abeokuta, Southwerstern Nigeria. *Global J Pharmacol*; 3(2): 69-80.

Antibiotics Sensitivity And Resistance Patterns Of Uropathogens To Nitrofurantoin And Nalidixic Acid In Pregnant Women With Urinary Tract Infections in ibadan, nigeria. Middle-east. *J. Sci. Res*; 4(2): 105-109.

Omoigberale, M.N.O., Iyamu, M.I., Amengialue, O.O., Egharevba A.P. and Edobor, O. (2014). Antibiotic resistance pattern of Staphylococcus aureus isolated from high vaginal swab and urethral swab specimens. *American Journal of Research Communication*; 2(1): 96-107.

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Otajevwo, F.D. and Momoh, S.A. (2013). Resistance marker loss of multi-drug resistant (MDR) Staphylococcus aureus strains after treatment with dilutions of acridine orange. *Journ. App.Med. Sci*; 2(2): 43-62.

Oteo, J., Campos, J. and Baquero, F. (2002). Antibiotics sensitivity resistance in 1962 invasive isolates of Escherichia coli in 27 spanish hospitals participating in the European Antimicrobial Resistance Surveliance System. *J. Antimicrobe. Chemother;* 50: 945-952.

Paterson D. L. and Bonomo R. A. (2005). Extended-spectrum β-Lactamases: a clinical update. *Clin. Microbiol. Rev*; 18: 657–68610.

Poirel L., Pitoud J. D., Nordmann P. (2007). Carbapenemases: molecular diversity and clinical consequences. *Food Microbiol*; 2: 501–512.

Pratibha S., Renuka S.W, Ninad V.P, Hemalata M.P, Sheetal S.J. and Prashant, K.D. (2015). *Int J Pharm Pharm Sci*; 7 (4): 298-301.

Radha, K.., Mahima, R., Ramanathan, G. and Thangapandian, V. (2012). Survey on drug Resistance pattern of Clinical Isolates and Effect of Plant Extract on the Drug Resistance Pattern. ISCA. *J. Biological Sc*i; 14-19.

Sahm, D.F., Thornsberry, C., Maryfield, D.C., Jones, M.E. and Karlowsky. J.A. (2001). Multi drug-resistance urinary tract isolates of Escherichia coli: prevalence and patient demographics in the United States. *Antimicrob. Agents Chemother*; 45:1402-1406.

Salisbury, V., Hedges, R.W. and Datta, N. (1972). Two modes of 'curing' transmissible plasmids. *J. Gen. Microbiol*; 70: 443-452.

Segen, J.C. (2012). Medical Dictionary. Farlex incorporation.

Shanahan P.M.A., Thomson, C.J. and Amyer, (1994). The global impact of antibiotic resistant bacteria. Their sources and reservoirs. *Review of Medical Bacteriology*; 5:174.

Shoemaker, N.B., Wang, G.R. and Salyers, A.A. (1992). Evidence for natural transfer of a tetracycline resistance gene between bacteria from the human colon and bacteria from the bovine lumen. *Appl. Environ. Microbiol*; 58: 1313-1320.

Slama, T. G., Amin A., Brunton S. A. (2005). A clinician's guide to the appropriate and accurate use of antibiotics: The Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria. *Am. J. Med*; 1181–1186.

Southhamer, A.H., De Haan, P.G. and Bulten, E.J. (1963) Kinetics of F-curing by acridine orange in relation to the number of F-particles in Escherichia coll. *Genet. Res. Camb*; 4: 305-317.

Tomoeda, M., Inuzuka, M., Kubo, N. and Nakamura, S. (1968). Effective elimination of drug resistance and sex factors in Escherichia coli by sodium dodecyl sulfate. *J. Bacteriol*; 95:1078-1089.

Tula, M.Y. and Iyoha, O (2014). Incidence and Distribution of Multi-Drug Resistant Pathogens from Clinical Samples in a Tertiary Hospital in South-South, Nigeria. *Afr. J. Clin. Exper. Microbiol*; 15(3): 130-137.

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Wechsler, J. and Kline, B.C. (1980) Mutation and identifi- cation of the F-plasmid locus determining resistance to acridine orange curing. *Plasmid*; 4: 276-280.

Yah, C.S. and Eghafona, N.O. (2008). Plasmids: a vehicle for rapid transfer of antibiotic resistance markers of Salmonella species in animals. *International Journal of Integrative Biology*; 2(1): 55-61.

Yah, S.C., Eghafona, N.O. and Forbi, J.C. (2008). Plasmid-borne antibiotics resistance markers of Serratia marcescens: an increased prevalence in HIV/AIDS patients. *Sci. Res. Essay*; 3(1): 28 – 34.

Zaman, M.A., Pasha, M.H. and Akhter, M.Z. (2010). Plasmid Curing of Escherichia coli Cells with Ethidium Bromide, Sodium Dodecyl Sulfate and Acridine Orange. *Bangladesh J Microbiol*; 27(1):28-31.

AUTHOR'S CONTRIBUTIONS

Inyang, N. J.: Research idea and design
Omokhodion O. M.: Sample collection
Babatope I.O.: Drafted the work
Adewuyi G.M.: Approval for use of isolates
Olagboye J.A.: Sample Analysis
Ikheloa J.: Analysis of result
Umoh N.O.: Statistical analysis

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