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Original Article **Open Access**

Antibacterial activity and time kill kinetics of Amlodipine, Thioridazine and Promethazine against pathogenic clinical bacterial isolates

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Abstract:

Background: The emergence of multi-drug resistant bacterial strains worldwide has necessitated the scientific search for novel, potent, and affordable antimicrobial agents including medicinal plants and non-antibiotic drugs for therapy of infectious diseases. The objective of this study is to assess *in vitro* antibacterial activities and time kill kinetics of some non-antibiotic drugs against pathogenic clinical bacterial isolates.
Methodology: *In vitro* antibacterial activities including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time kill kinetics of Amlodipine (AML), Thioridazine (THI) and Promethazine (PRO) against *Staphylococcus aureus*, coagulase negative staphylococci (CoNS), *Streptococcus* spp, *Escherichia coli*, *Enterobacter* spp, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates were determined using disc diffusion, broth microdilution and plate count techniques.
Results: The mean growth inhibition zones by the disc diffusion assay of AML, THI and PRO against the isolates were ≤15.1±1.0 mm with MIC and MBC values ranging from 12.5 to 50µg/ml and 25 to 100µg/ml respectively. The time-kill assay revealed bactericidal effect of AML, THI and PRO on Gram positive bacteria evidenced by mean log reductions in viable bacterial cell counts ranging from 0.13 Log_{10} to 2.41 Log_{10} CFU/ml for *S. aureus*, 0.88 Log_{10} to 2.08 Log_{10} CFU/ml for *Streptococcus* spp, and 0.26 Log_{10} to 2.34 Log_{10} CFU/ml for CoNS after ≤30hrs post inoculation at 1xMIC. The range of log reduction in viable cell counts of Gram-negative bacteria exposed to AML, THI and PRO were *E. coli* (0.11 to 3.23 Log_{10} CFU/ml), *P. aeruginosa* (0.52 to 2.56 Log_{10} CFU/ml), *K. pneumoniae* (0.85 to 3.0 Log_{10} CFU/ml) and *Enterobacter* spp (0.38 to 2.08 Log_{10} CFU/ml) after ≤30 hrs post inoculation at 1x MIC.
Conclusion: These findings demonstrate *in vitro* antibacterial efficacies and time kill kinetics of AML, THI and PRO against pathogenic clinical bacterial isolates, which indicate that these non-antibiotic drugs may be useful therapeutic alternatives in the bid to reduce the burden of infectious diseases associated with antibiotic resistant pathogens.

Keywords: Amlodipine, Thioridazine, Promethazine, Time-Kill, Kinetics, MIC, MBC, bacteria

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Activité antibactérienne et cinétique de destruction du temps de l'amlodipine, de la thioridazine et de la prométhazine contre les isolats bactériens cliniques pathogènes

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Abstrait:

Contexte: L'émergence de souches bactériennes multirésistantes dans le monde a rendu nécessaire la recherche scientifique d'agents antimicrobiens nouveaux, puissants et abordables, notamment des plantes médicinales et des médicaments non antibiotiques pour le traitement des maladies infectieuses. L'objectif de cette étude est d'évaluer les activités antibactériennes in vitro et la cinétique de destruction temporelle de certains médicaments non antibiotiques contre les isolats bactériens cliniques pathogènes.
Méthodologie: activités antibactériennes *in vitro*, y compris la concentration minimale inhibitrice (CMI), la concentration bactéricide minimale (MBC) et la cinétique de destruction du temps de l'amlodipine (AML), de la thioridazine (THI) et de la prométhazine (PRO) contre *Staphylococcus aureus*, les staphylocoques à coagulase négative (CoNS), *Streptococcus* spp, *Escherichia coli*, *Enterobacter* spp, *Klebsiella pneumoniae* et *Pseudomonas aeruginosa* ont été déterminés en utilisant des techniques de diffusion sur disque, de microdilution en bouillon et de numération sur plaque.
Résultats: Les zones moyennes d'inhibition de la croissance par le test de diffusion de disque d'AML, THI et PRO contre les isolats étaient ≤15,1±1,0mm avec des valeurs MIC et MBC allant de 12,5 à 50μg/ml et de 25 à 100μg/ml respectivement. Le dosage temporel a révélé un effet bactéricide de la LMA, du THI et du PRO sur les bactéries Gram positives, mis en évidence par des réductions logarithmiques moyennes du nombre de cellules bactériennes viables allant de 0,13 Log10 à 2,41 Log10 CFU/ml pour *S. aureus*, 0,88 Log10 à 2,08 Log10 CFU/ml pour *Streptococcus* spp et 0,26 Log10 à 2,34 Log10 CFU/ml pour CoNS après ≤ 30 heures après l'inoculation à 1 x MIC. La plage de réduction logarithmique du nombre de cellules viables de bactéries à Gram négatif exposées à la LMA, au THI et au PRO était *E. coli* (0,11 à 3,23 Log10 CFU/ml), *P. aeruginosa* (0,52 à 2,56 Log10 CFU/ml), *K. pneumoniae* (0,85 à 3,0 Log10 CFU/ml) et *Enterobacter* spp (0,38 à 2,08 Log10 CFU/ml) après ≤ 30 heures après l'inoculation à 1 x MIC.
Conclusion: Ces résultats démontrent une efficacité antibactérienne *in vitro* et une cinétique de destruction du temps des LMA, THI et PRO contre les isolats bactériens cliniques pathogènes, ce qui indique que ces médicaments non antibiotiques peuvent être des alternatives thérapeutiques utiles dans le but de réduire le fardeau des maladies infectieuses associées aux antibiotiques pathogènes résistants.

Mots-clés: Amlodipine, Thioridazine, Prométhazine, Time-Kill, Cinétique, MIC, MBC, bactéries

Introduction:

Although the pharmaceutical industries are still in the business of producing new antibiotics for treatment of infectious diseases, the numbers of infections caused by resistant microorganisms continue to increase owing to combinations of microbial characteristics and selective pressure created by antimicrobial use (1,2). The mechanisms of microbial resistance to antimicrobial agents include the production of structure-inactivating enzymes, alteration of cell wall, cell membrane and enzyme (e.g. DNA gyrase) target sites and ribosomal modification (3). The emergence of multi-drug resistant (MDR) microbial strains has necessitated the scientific search for potent, novel and affordable antimicrobial agents including plants (4,5) and non-antibiotic drugs for the treatment of infectious diseases.

Scores of non-antibiotic drugs, pharmaceutical preparations belonging to different pharmacological classes, currently used in the treatment of non-infectious diseases have been reported to exhibit both *in vitro* and *in vivo* antimicrobial activities (6). The non-antibiotic drugs, which inhibit microbial metabolism (7), with either biocidal or biostatic activity include anti-psychotics (thioridazine, clozapine), anti-hypertensives (nifedipine and amlodipine) (8), non-steroidal anti-inflammatory drugs (ibuprofen, diclofenac sodium) (9), pump inhibitors (esomeprazole, omeprazole), anti-histamines (promethazine, trimeprazine) (10), and anti-depressants (sertraline and paroxetine) (11).

Promethazine is usually administered to attenuate inflammatory process in disease conditions such as allergic rhinitis, conjunctivitis, and urticaria (12,13). The use of anti-histamines, especially the first-generation phenothiazine H1-inhibitors such as promethazine for patients with microbial infection may become inevitable and this has prompted the evaluation of their antimicrobial properties.

The antibacterial activity of amlodipine, a cardiovascular drug, was reported by Kumar et al., (8) on several clinical bacterial isolates including *Staphylococcus aureus*, *Escherichia coli*, *Vibrio* spp, *Salmonella* spp, *Bacillus* spp and *Pseudomonas aeruginosa.* Similarly, in addition to the antipsychotic properties of thioridazine, significant antimicrobial activities against intracellular microorganisms have been reported (14). The objective of this study is to

determine the antibacterial activities and time kill kinetics of amlodipine, thioridazine and promethazine against selected pathogenic clinical bacterial isolates.

Materials and method:

Identification of bacterial isolates

Fourteen (14) clinical bacterial isolates comprising *Staphylococcus aureus* (n=2), coagulase negative staphylococci (n=2), *Streptococcus* spp (n=2), *Escherichia coli* (n=2), *Klebsiella pneumoniae* (n=2), *Enterobacter* spp (n= 2), and *Pseudomonas aeruginosa* (n=2) were obtained from the Department of Microbiology, University of Uyo, Akwa Ibom State, Nigeria. These bacteria were isolated from clinical specimens and identified using conventional morphological and biochemical tests (15).

Source of the non-antibiotic drugs (Amlodipine, Thioridazine and Promethazine)

Amlodipine (AML, MAF India Pharmaceutical, India), Thioridazine (THI, Indian Generic Company, India) and Promethazine (PRO, CSC Pharmaceuticals International, India) were purchased in tablet forms from registered Pharmacy stores in Uyo. Stock solution of each drug was prepared by dissolving 1g of each in 100ml of sterile distilled water to give a concentration of 10mg/ml. The stock solution was further diluted to produce 5mg/ml and 2.5 mg/ml concentrations for each of AML, THI and PRO.

Determination of antibacterial activity of the non-antibiotic drugs by disc diffusion assay

The antibacterial activities of AML, THI and PRO on the clinical bacterial isolates were determined by the disc diffusion method (16). Paper discs of AML, THI and PRO were prepared by punching sterile Whatman filter paper (No.1) with a 6 mm disc puncher and sterilizing them at 160°C for one hour. Each disc was then impregnated with 10µL of 2.5mg/ml and 5.0mg/ml concentrations of AML, THI and PRO to give disc contents of 25µg and 50µg respectively.

Mueller-Hinton agar (MHA) plates were aseptically prepared and 0.1ml of each bacterial inoculum, prepared directly from an overnight nutrient agar plate and adjusted to 0.5 McFarland standards, was inoculated using sterile pipette onto each of the MHA plate. The impregnated discs were carefully placed on the MHA plates and incubated aerobically at 37°C for 24 hours followed by measurement of the diameters of zone of inhibition (in millimetres) using a calibrated ruler. The disc diffusion assays were performed in triplicates and the mean diameters of zones of inhibition were recorded.

Determination of minimum inhibitory (MIC) and minimum bactericidal concentration (MBC)

The MICs of AML, THI and PRO against the bacterial isolates were determined using macrobroth dilution technique in test tubes (5). One ml of the stock solution (10mg/ml) of AML, THI and PRO was serially diluted in test tubes to obtain concentrations of 5, 2.5, 1.25 and 0.625mg/ml for each of the non-antibiotic solution. To 0.1ml (100µL) of each of these concentrations was added 9.9ml nutrient broth (1 in 100 dilution) to give the final concentrations of AML, THI and PRO of 100, 50, 25, 12.5 and 6.25µg/ml for the MIC testing. A loopful of prepared inoculum of each bacterium was added to each of the non-antibiotic solution. A tube containing only nutrient broth was inoculated with bacterial isolate to serve as control. All culture tubes were incubated aerobically at 37°C for 24 hours, after which the tubes were examined for microbial growth by observing for turbidity. The MIC was read as the least concentration of the non-antibiotic solution that visibly inhibited the growth of the test bacterial isolate after 24 hours incubation.

For the MBC, an aliquot of 1ml from each of the MIC broth tubes that showed no visible growth was streak-inoculated onto sterile nutrient agar plates using a sterile pipette. The inoculated plates were inverted and incubated at 37°C aerobically for 24 hours. The MBC values of AML, THI and PRO were considered as the concentration of each non-antibiotic solution that resulted in killing of the bacterial isolates, which showed as no visible colonies on the agar plates.

Time-kill analysis

The time-kill analysis was carried out using macrobroth dilution and pour plate techniques. An overnight nutrient broth culture of each bacterial isolate in test tube was adjusted to 0.5 McFarland turbidity standard to obtain a starting inoculum of between 10^5 and 10^6 CFU/ml (confirmed by quantitative plate counts). The tubes were incubated at 37°C with shaken at 150 rpm for 90 mins to ensure that microbial growths were in the logarithmic (exponential) phase. One (1) millilitre of this exponentially growing bacterial cultures was added to 9ml of MHB containing MIC concentration (1ml) of AML, THI and PRO. Bacterial growth was quantified at time '0' hour and at

6, 12, 18, 24, and 30 hours of incubation by aseptically taking 1ml of the aliquot, diluting serially (10-fold dilutions) in sterile normal saline and plating out 1ml of the final dilution onto nutrient agar plates. The plates were incubated aerobically for 24 hours at 37°C after which the colonies on each plate were enumerated and viable cells expressed as CFU/ml. Inoculated medium without AML, THI and PRO was also set up and plated on nutrient agar as control. All experiments were performed in triplicates.

The percentage and logarithm reductions of the bacterial cells exposed to AML, THI and PRO were calculated for each of the time intervals. The Log_{10} CFU/ml of survived bacterial cells against exposure time (in hours) were plotted on a semi-logarithm graph for each bacterial pathogen to obtain the time–kill curve. Activity of the non-antibiotic drugs was considered bactericidal at the lowest concentration that reduced the initial inoculum by $>3Log_{10}$ CFU/ml (99.9% killing). The percentage and logarithm reductions of the bacterial cells exposed to AML, THI and PRO were respectively calculated as; percentage reduction = (initial counts – counts at 'x' interval)/ initial counts (multiply by 100), while the logarithm reduction = Log_{10} (initial counts) – Log_{10} (counts at 'x' interval).

Statistical analysis

All experiments were performed in triplicates and data analysis was done with the Statistical Package for the Social Sciences (SPSS) version 20.0. Data were presented as mean ± SD and comparison between mean values was done using the Duncan multiple range test, with significance level at $p<0.05$.

Results:

The mean growth inhibitory zone of AML against Gram-positive bacteria (GPB) ranged from 10.1±0.2mm (25μg disk) to 13.6 ±0.5mm (50μg disk), and against Gram-negative bacteria (GNB), from 10.8±0.2mm (25μg disk) to 14.5±0.5mm (50μg disk). The mean growth inhibitory zone of THI against GPB ranged from 8.8±0.1mm (25μg disk) to 14.1 ±0.5mm (50μg disk) and against GNB, from 10.2±0.2mm (25μg disk) to 14.7±1.0mm (50 μg disk). For PRO, the mean growth inhibitory zone ranged from 9.3±0.1mm (25μg disk) to 14.5±0.3mm (50μg disk) against GPB, and 8.3 ±0.1 mm (25 μg disk) to 15.1±1.0mm (50μg) against GNB. The mean growth inhibitory zone of the levofloxacin control disk (30μg) was

Table 1: Antibacterial activities of non-antibiotic drugs on bacterial pathogens with disk diffusion method

Bacterial Isolates	Isolates Code	Zone of Inhibition in mm (mean±SD)						Control (Levofloxacin) (30μg disk)
		Amlodipine		Thioridazine		Promethazine		
		2.5mg/ml (25μg disk)	5mg/ml (50μg disk)	2.5mg/ml (25μg disk)	5mg/ml (50μg disk)	2.5mg/ml (25μg disk)	5mg/ml (50μg disk)	
Staphylococcus aureus	SA01	$11.2±0.1^a$	$13.6±0.5^b$	$11.6±0.1^a$	$14.1±0.5^b$	$12.3±0.2^b$	$14.5±0.3^b$	$13.6±0.3^a$
Staphylococcus aureus	SA02	NZ	$9.8±0.2^a$	NZ	$9.3±0.1^a$	NZ	NZ	NZ
CoNS	CS02	NZ	$10.2±0.1^a$	$9.0±0.1^a$	$13.6±0.4^b$	$9.3±0.1^a$	$13.0±0.2^b$	$14.5±0.5^a$
CoNS	CS01	NZ	$12.4±0.3^b$	$11.2±0.3^a$	$14.0±0.2^b$	NZ	$11.6±0.2^a$	$14.0±0.1^a$
Streptococcus spp	SS01	$10.1±0.2^a$	$13.0±0.5^b$	$10.0±0.1^a$	$12.7±0.2^b$	$10.4±0.2^a$	$12.1±0.5^b$	$15.6±0.3^b$
Streptococcus spp	SS02	NZ	$9.1±0.1^a$	$8.8±0.1^a$	$10.4±0.1^a$	$9.6±0.1^a$	$11.2±0.1^a$	$14.3±0.2^a$
Escherichia coli	EC01	$12.9±0.3^b$	$14.5±0.5^c$	$10.5±0.2^b$	$13.1±0.3^b$	$11.3±0.3^a$	$13.9±0.5^b$	$15.1±0.5^b$
Escherichia coli	EC02	$12.2±0.1^b$	$14.0±0.3^b$	$11.9 ±0.1^a$	$13.8±0.3^b$	$11.5±0.2^a$	$13.0±0.5^b$	NZ
Klebsiella pneumoniae	KP01	NZ	$10.9±0.5^a$	$10.2±0.2^a$	$11.5±0.1^a$	NZ	$12.4±0.2^b$	NZ
Klebsiella pneumoniae	KP02	$11.5±0.5^a$	$13.7±1.0^b$	$12.1±0.5^b$	$14.7±1.0^c$	$11.0±0.5^a$	$12.9±0.5^b$	$16.0±1.0^b$
Enterobacter spp	ES02	$12.0±0.2^b$	$14.4±1.0^b$	$12.6±0.5^b$	$14.7±0.5^c$	$12.6±0.2^b$	$15.1±1.0^c$	$16.5±0.5^b$
Enterobacter spp	ES01	$10.8±0.2^a$	$12.9±0.2^b$	$10.8±0.1^a$	$13.3±0.2^b$	NZ	$11.3±0.2^a$	NZ
Pseudomonas aeruginosa	PA01	$12.4±0.5^b$	$13.6±0.5^b$	$11.6±0.2^a$	$12.9±0.3^b$	$12.1±0.2^b$	$13.7±0.5^b$	$14.8±0.5^a$
Pseudomonas aeruginosa	PA02	NZ	$8.6±0.1^a$	NZ	$9.5±0.1^a$	$8.3±0.1^a$	$10.9±0.2^a$	$12.0±0.3^a$

CONS = Coagulase negative staphylococci; SD = Standard deviation; NZ = No inhibitory zone. Each value represents the mean plus standard deviation of three replicates. Mean within the column followed by different superscript letters are significant by Duncan's multiple range test ($p<0.05$)

Table 2: Minimum inhibitory and minimum bactericidal concentrations of non-antibiotic drugs against bacterial pathogens

Bacterial Isolates	Isolate Codes	MIC/MBC (µg/ml) AML	THI	PRO
Staphylococcus aureus	SA01	12.5/25	12.5/25	12.5/25
Staphylococcus aureus	SA02	50/> 50	50/> 50	> 50/100
CoNS	CS02	50/> 50	25/50	25/50
CoNS	CS01	50/50	12.5/25	50/50
Streptococcus spp	SS01	25/50	25/50	12.5/50
Streptococcus spp	SS02	50/> 50	25/> 50	25/> 50
Escherichia coli	EC01	12.5/50	25/> 50	12.5/50
Escherichia coli	EC02	12.5/25	12.5/25	12.5/25
Klebsiella pneumoniae	KP01	50/50	25/50	50/> 50
Klebsiella pneumoniae	KP02	25/> 50	12.5/25	25/50
Enterobacter spp	ES02	12.5/25	12.5/25	12.5/50
Enterobacter spp	ES01	25/> 50	25/50	50/> 50
Pseudomonas aeruginosa	PA01	12.5/> 50	12.5/50	12.5/25
Pseudomonas aeruginosa	PA02	50/> 50	50/> 50	25/50

CoNS = Coagulase negative staphylococci; MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration; AML = Amlodipine; THI = Thioridazine; PRO = Promethazine

13.6±0.3 to 15.6±0.3 mm against GPB, and 12.0±0.3 to 16.5±0.5 mm against GNB (Table 1).

The AML, THI and PRO inhibited the growth of all the 14 isolates tested with MIC values in the range of 12.5 – 50 µg/ml, 12.5 - 50µg/ml and 12.5 - >50µg/ml respectively. The MIC values of THI were lowest for *S. aureus*, *E. coli*, *K. pneumoniae*, *Enterobacter* spp, *P. aeruginosa* and CoNS*.* The MBC values of AML, THI and PRO were in the range of 25 - 100 µg/ ml. The MBC of AML for 78.6% of the bacterial isolates tested was 50 µg/ml, the MBC of THI for 35.7% of bacterial isolates tested was 25 µg/ml while the MBC of PRO for 7.1% of the bacterial isolates tested was 100 µg/ml (Table 2). The results also indicated that both MIC and MBC end points obtained by visual reading for AML on CoNS and *K. pneumoniae* were equal.

The bactericidal activity was deemed to be present if there was a ≥ 99.9% reduction in survival from the original inoculum (≥99.9% killing). Table 3 showed the percentage and log reductions in GPB cells exposed to AML, THI and PRO at 6 hours intervals after incubation. The percentage and log reduction in viable cell counts of *S. aureus* exposed to AML ranged from 33.3 to ≥ 99.9% and 0.18 to 2.41 Log_{10} CFU/ml after 30 hrs of interaction respectively while the percentage and log reduction in viable cell counts of *Streptococcus* spp exposed to AML ranged from 86.96 to ≥ 99.9% and 0.88 to 2.08 Log_{10} CFU/ml after 30 hours of interaction respectively.

The lowest percentage and logarithm

Table 3: Percentage and logarithm reductions in Gram positive bacterial cells exposed to non-antibiotic drugs

Isolate Codes	Exposed Time (hr)	Amlodipine PC (CFU/ml)	Log 10 CFU/ml	% / Log Reduction	Thioridazine PC (CFU/ml)	Log 10 CFU/ml	% / Log Reduction	Promethazine PC (CFU/ml)	Log 10 CFU/ml	% / Log Reduction
SA01	0	3.2×10^5	5.51	NA / NA	3.6×10^5	5.56	NA /NA	3.2×10^5	5.51	NA / NA
	6	4.5×10^4	4.65	33.33 / 0.18	2.4×10^4	4.38	93.33 /1.18	4.5×10^4	4.65	85.90 / 0.86
	12	1.3×10^3	3.11	91.67 /1/08	2.2×10^3	3.34	90.83 / 1.04	1.3×10^3	3.11	97.11 / 1.54
	18	2.7×10^2	2.43	83.50 / 0/78	1.7×10^2	2.23	92.27 / 1.11	2.7×10^2	2.43	79.23 / 0.68
	24	2.0×10^2	2.30	92.12 /1.10	NG	0.0	≥99.9 / 2.23	2.0×10^2	2.30	25.93 / 0.13
	30	1.2×10^2	2.08	≥99.9/ 2.41	NG	0.0	≥99.9 / 0.0	1.2×10^2	2.08	40.00 /0.22
SS02	0	3.6×10^5	5.56	NA / NA	4.8×10^5	5.68	NA / NA	5.5×10^5	5.74	NA / NA
	6	2.3×10^4	4.36	93.61 /1.20	2.4×10^4	4.38	95.0 /1.30	2.4×10^4	4.38	95.64 /1.36
	12	3.0×10^3	3.48	86.96 / 0.88	1.0×10^3	3.00	95.83 / 1.38	3.3×10^3	3.52	86.25 / 0.86
	18	1.2×10^2	2.08	96.00 / 1.40	1.2×10^2	2.08	88.00 / 0.92	2.2×10^2	2.34	93.33 / 1.18
	24	NG	0.0	≥99.9 /2.08	NG	0.0	≥99.9 /2.08	1.2×10^2	2.08	45.50 / 0.26
	30	NG	0.0	≥99.9 / 0.0	NG	0.0	≥99.9 /0.0	NG	0.0	≥99.9 / 2.08
CS02	0	4.8×10^5	5.68	NA / NA	3.6×10^5	5.56	NA / NA	3.6×10^5	5.56	NA / NA
	6	3.6×10^4	4.56	92.50 / 1.12	7.2×10^4	4.86	80.0 /0.70	2.0×10^5	5.30	44.44 / 0.26
	12	2.9×10^3	3.46	91.94 /1.10	5.3×10^3	3.72	92.64 / 1.14	3.6×10^4	4.56	82.00 / 0.74
	18	1.2×10^3	3.08	58.62 / 0.38	3.0×10^3	3.48	43.40 / 0.24	2.0×10^3	3.30	94.44 / 1.26
	24	2.0×10^2	2.30	83.33 / 0.78	2.2×10^2	2.34	92.67 / 1.14	1.6×10^2	2.20	92.00 / 1.10
	30	NG	0.0	≥99.9 /2.30	NG	0.0	≥99.9 / 2.34	NG	0.0	≥99.9 / 2.20

SA01 = *Staphylococcus aureus*; SS02 = *Streptococcus* spp; CSO2 = Coagulase negative staphylococcus; PC = Plate Counts; CFU = Colony Forming Units; ml = Millilitre; NG = No Growth; NA = Not Available

Table 4: Percentage and logarithm reductions in Gram negative bacterial cells exposed to non-antibiotic drugs

Isolate Codes	Exposed Time (hr)	Amlodipine			Thioridazine			Promethazine		
		PC (CFU/ml)	Log 10 CFU/ml	% / Log Reduction	PC (CFU/ml)	Log 10 CFU/ml	% / Log Reduction	PC (CFU/ml)	Log 10 CFU/ml	% / Log Reduction
EC01	0	3.6×10^5	5.56	NA / NA	6.9×10^5	5.84	NA / NA	3.9×10^5	5.59	NA / NA
	6	7.2×10^4	4.86	80.00 / 0.70	7.0×10^4	4.85	89.85 / 0.99	2.4×10^4	4.38	93.85 / 1.21
	12	6.3×10^3	3.80	91.25 / 1.06	2.4×10^3	3.38	96.57 / 1.47	2.2×10^3	3.34	90.83 / 1.04
	18	2.0×10^2	2.30	96.83 / 1.50	2.3×10^2	2.36	90.42 / 1.02	1.7×10^2	3.23	92.27 /0.11
	24	NG	0.0	≥99.9 /2.30	NG	0.0	≥99.9 / 2.36	NG	0.0	≥99.9 / 3.23
	30	NG	0.0	≥99.9 / 0.0	NG	0.0	≥99.9 / 0.0	NG	0.0	≥99.9 / 0.0
ES02	0	1.9×10^5	5.28	NA / NA	2.4×10^5	5.38	NA / NA	2.0×10^5	5.30	NA / NA
	6	1.7×10^4	4.23	91.05 / 1.05	1.0×10^5	5.00	58.33 / 0.38	2.4×10^4	4.38	88.00 / 0.92
	12	1.2×10^3	3.08	92.94 / 1.15	1.5×10^4	4.18	85.00 / 0.82	1.2×10^4	4.08	50.00 / 0.30
	18	2.4×10^2	2.38	80.00 / 0.70	2.4×10^3	3.38	84.00 /0.80	3.6×10^3	3.56	70.00 / 0.52
	24	1.0×10^2	2.00	58.33 / 0.38	1.2×10^2	2.08	95.00 /1.30	2.2×10^2	2.34	93.89 / 1.22
	30	NG	0.0	≥99.9 /2.00	NG	0.0	≥99.9 /2.08	1.0×10^2	2.00	54.55 / 0.34
KP02	0	3.9×10^5	5.59	NA / NA	3.9×10^5	5.59	NA / NA	3.3×10^5	5.52	NA / NA
	6	2.4×10^4	4.38	93.85 /1.21	2.4×10^4	4.38	93.85 / 1.21	3.0×10^4	4.48	90.91 / 1.04
	12	1.2×10^3	3.08	95.00 /1.30	2.2×10^3	3.34	90.83 / 1.04	1.0×10^3	3.00	96.67 / 1.48
	18	1.7×10^2	2.23	85.83 /0.85	1.7×10^2	2.23	92.27 / 1.02	NG	0.0	≥99.9 / 3.00
	24	NG	0.0	≥99.9 /2.23	NG	0.0	≥99.9 / 2.23	NG	0.0	≥99.9 / 0.0
	30	NG	0.0	≥99.9/ 0.0	NG	0.0	≥99.9 / 0.0	NG	0.0	≥99.9 / 0.0
PA01	0	7.7×10^5	5.89	NA / NA	2.0×10^5	5.30	NA / NA	4.8×10^5	5.68	NA / NA
	6	2.0×10^5	5.30	74.03 / 0.59	1.4×10^4	4.15	93.00 / 1.15	2.4×10^4	4.38	95.00 / 1.30
	12	1.2×10^4	4.08	94.00 /1.22	1.2×10^3	3.08	91.43 / 1.07	1.0×10^3	3.00	95.83 / 1.38
	18	3.3×10^3	3.52	72.50 / 0.56	3.6×10^2	2.56	70.00 / 0.52	1.2×10^2	2.08	88.00 / 0.92
	24	2.0×10^2	2.30	93.94 /1.22	NG	0.0	≥99.9 / 2.56	NG	0.0	≥99.9 / 2.08
	30	NG	0.0	≥99.9 /2.30	NG	0.0	≥99.9 / 0.0	NG	0.0	≥99.9 / 0.0

EC01 = *Escherichia coli*; ES02 = *Enterobacter* spp; KP02 = *Klebsiella pneumoniae*; PA01 = *Pseudomonas aeruginosa*; PC = Plate Counts; CFU = Colony Forming Units; ml = Millilitre; NG = No Growth; NA = Not Available

reduction in viable cell count of CoNS exposed to AML was ≥ 58.6% and 0.38 Log_{10} CFU/ml respectively. The log reduction in viable cell counts of *S. aureus, Streptococcus* spp and CoNS exposed to PRO for 30 hrs ranged from 0.13 to 1.54 Log_{10} CFU/ml, 0.26 to 2.08 Log_{10} CFU/ml and 0.26 to 2.20 Log_{10} CFU/ml respectively (Table 3). At 1xMIC, THI achieved bactericidal effects on *S. aureus, Streptococcus* spp, *E. coli, K. pneumoniae* and *P. aeruginosa* at 24 hours post inoculation while ≥ 99.9% reduction in survival from the original inoculum was achieved for CoNS and *Enterobacter* spp at 30 hours post inoculation (Tables 3 and 4).

The ranges of log reduction in viable cell counts of GNB exposed to AML for 30 hrs were *E. coli* (0.7 to 2.30 Log_{10} CFU/ml), *Enterobacter* spp (0.38 to 2.0 Log_{10} CFU/ml), *K. pneumoniae* (0.85 to 2.23 Log_{10} CFU/ml) and *P. aeruginosa* 0.56 to 2.3 Log_{10} CFU/ml) (Table 4). The bactericidal activity of PRO on *K. pneumoniae* and *P. aeruginosa* was achieved at 18- and 30-hours post inoculation respectively whereas 1.0×10^2 CFU/ml *Enterobacter* spp were still viable at 30 hours post inoculation (Table 4). The time kill kinetics curves of AML, THI and PRO (1.0 x MIC) against the bacterial isolates are shown in Figs 1 and 2.

The increase in viable cell counts of the GPB and GNB not exposed to AML, THI and PRO within the 30 hours of incubation were observed and presented in Tables 5 and 6. The viable cell count of *S. aureus* increased from 5.57 to 7.79 Log_{10} CFU/ml, those of S*trepto-coccus* spp cells increased from 5.61 to 7.85 Log_{10} CFU/ml while those of CoNS increased from 5.61 to 7.85 Log_{10} CFU/ml (Table 5). The increase in the viable cell count of GNB within the 30 hours incubation period was 5.60 to 7.84 Log_{10} CFU/ml for *E coli,* 5.41 to 7.60 Log_{10} CFU/ml for *Enterobacter* spp, 5.58 to 7.72 Log_{10} CFU/ml for *K. pneumoniae* and 5.76 to 7.86 Log_{10} CFU/ml for *P. aeruginosa* (Table 6).

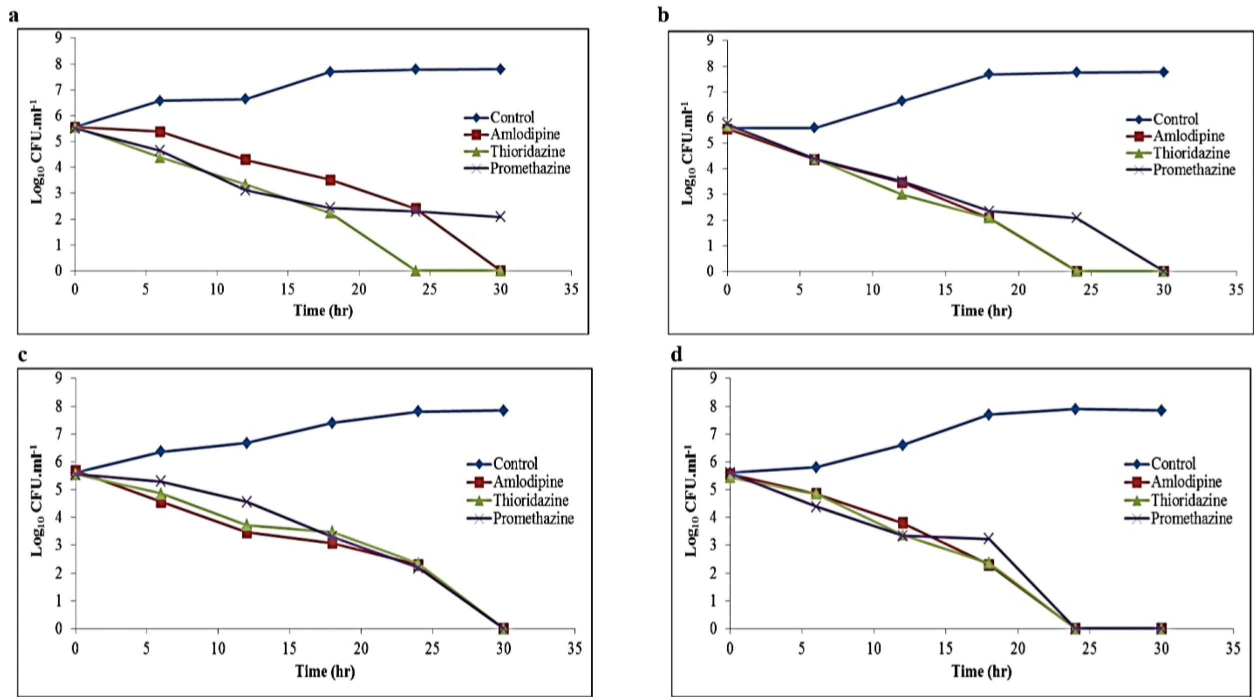

Fig 1: Time Kill Kinetics Curve of Amlodipine, Thioridazine and Promethazine (1 x MIC) and control against (a) *S. aureus,* (b) *Streptococcus* spp., (c) Coagulase negative staphylococci, (d) *Escherichia coli*

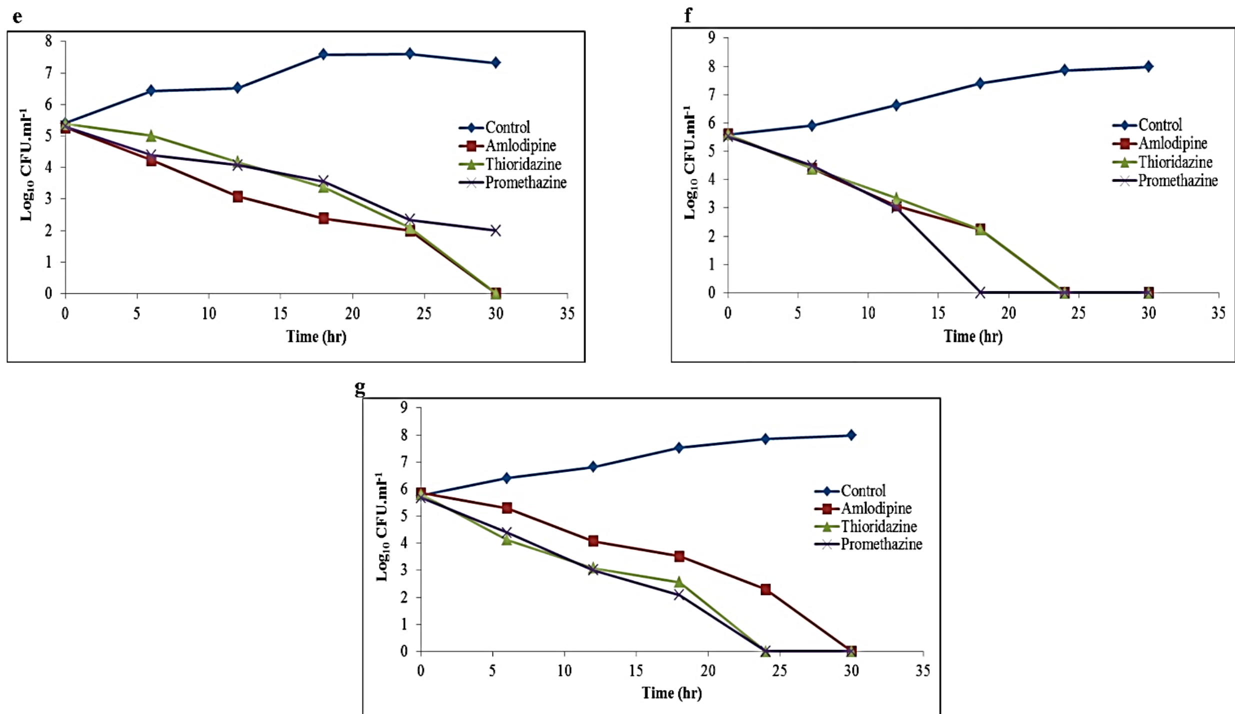

Fig 2: Time Kill Kinetics Curve of Amlodipine, Thioridazine, Promethazine (1 x MIC) and control against (e) *Enterobacter* spp., (f) *Klebsiella pneumoniae*, (g) *Pseudomonas aeruginosa*

Table 5: Growth of Gram-positive bacterial cells unexposed to non-antibiotic drugs

Bacterial Isolates	Codes	Time Interval (Hr)	Plate Counts (CFU/ml)	Log $_{10}$ CFU/ml
Staphylococcus aureus	SA01	0	3.7×10^5	5.57
		6	3.8×10^6	6.58
		12	4.4×10^6	6.64
		18	5.0×10^7	7.70
		24	6.1×10^7	7.78
		30	6.2×10^7	7.79
Streptococcus spp	SS02	0	3.9×10^5	5.59
		6	3.9×10^5	5.59
		12	4.4×10^6	6.64
		18	4.8×10^7	7.68
		24	5.7×10^7	7.76
		30	5.9×10^7	7.77
Coagulase negative *Staphylococcus*	CS02	0	4.1×10^5	5.61
		6	2.3×10^6	6.36
		12	4.7×10^6	6.67
		18	3.0×10^7	7.48
		24	6.4×10^5	7.81
		30	7.0×10^5	7.85

Table 6: Growth of Gram-negative bacterial cells unexposed to non-antibiotic drugs

Bacterial Isolates	Codes	Time Interval (Hr)	Plate Counts (CFU/ml)	Log $_{10}$ CFU/ml
Escherichia coli	EC01	0	4.0×10^5	5.60
		6	6.6×10^5	5.82
		12	4.1×10^6	6.61
		18	5.5×10^7	7.74
		24	7.9×10^7	7.90
		30	6.9×10^7	7.84
Enterobacter spp	ES02	0	2.6×10^5	5.41
		6	2.7×10^6	6.43
		12	3.3×10^6	6.52
		18	3.8×10^6	6.58
		24	4.3×10^7	7.63
		30	4.0×10^7	7.60
Klebsiella pneumoniae	KP02	0	3.8×10^5	5.58
		6	1.0×10^6	6.00
		12	4.3×10^6	6.63
		18	4.9×10^7	7.69
		24	5.1×10^7	7.71
		30	5.3×10^7	7.72
Pseudomonas aeruginosa	PA01	0	5.8×10^5	5.76
		6	6.0×10^6	6.78
		12	6.4×10^6	6.81
		18	6.9×10^7	7.82
		24	7.1×10^7	7.85
		30	7.3×10^7	7.86

Discussion:

The global emergence of multi-drug resistant pathogens as well as the continuing challenge of infectious diseases have necessitated the scientific exploration of novel, potent and affordable antimicrobial agents such as medicinal plants (5) and non-antibiotic drugs (6) in the management of infectious diseases. In this study, amlodipine (AML) demonstrated higher growth inhibitory activities at 5mg/ml concentration (50µg disk) against the GPB (*S. aureus,* CoNS, *Streptococcus* spp) and GNB (*E. coli, Enterobacter* spp, *K. pneumoniae* and *P. aeruginosa*) than at 2.5mg/ml concentration (25µg), indicating a concentration-dependent inhibition of bacterial growth.

The antibacterial activity of AML in this study substantiates the findings of Kumar et al., (8) that AML, a calcium channel blocker and cardiovascular drug, exhibited antibacterial activities against *S. aureus, E. coli* and *P. aeruginosa.* The antibacterial activities of AML on both GPB and GNB indicated its broad-spectrum activity and these findings concurs with the reports of Mazumdar et al., (17) and Pereira et al., (18), which reported broad-spectrum antibacterial activities of AML. The pattern of time-kill kinetics, investigated by the microbroth kinetic growth assay, against the GPB and GNB showed that AML exhibited bactericidal activities (≥ 99.9% reduction in

survival from the original inoculum) within 30 hours of exposure against the bacteria. These bactericidal activities of AML against the isolates in this study confirmed the findings previously reported by Kumar et al., (8) and Mazumdar et al., (17).

The MBC values of THI for the isolates ranged between 25 and 100µg/ml with bactericidal effects on *S. aureus, Streptococcus* spp, *E. coli, K. pneumoniae* and *P. aeruginosa* at ≤ 24 hours post inoculation. The antibacterial activities of THI on GPB and GNB in this study corroborates the findings of Radhakrishnan et al., (19) on the potentiality of THI as an effective antibacterial agent. Our findings on antibacterial efficacy of THI on *P. aeruginosa* was also in conformity with a previous study by Mukherjee et al., (20), who reported that THI, an anti-psychotic drug, was a highly promising agent in the treatment of acute infections caused by *P. aeruginosa.* Studies have shown that THI exerts a bactericidal effect on bacteria by damaging the cell wall and causing major changes in expression of many genes involved in peptidoglycan biosynthesis (21). THI has also been reported to reverse antibiotic resistance by facilitating the elimination of resistance (R) plasmid from MDR bacteria (14,19).

In this study, PRO also exhibited antibacterial activities on both GPB and GNB, with log reduction in viable cell counts of GPB exposed for 30 hours ranging from 0.13 to 2.20 Log_{10} CFU/ml. Our finding substantiates the reports of Dasgupta et al., (22) on antibacterial activity of PRO against antibiotic resistant bacterial isolates. Bactericidal activity of PRO against *S. aureus* in our study also agrees with the findings of Dastidar et al., (23). PRO, a phenothiazine, acts by inhibiting the efflux pump that protects bacterial cell against harmful chemical substances (24), and also affects energy sources, adenosine triphosphatase (ATPase), and genes that regulate permeability in bacteria (25). The increased permeability ensures that PRO molecules get to the DNA sites, intercalate between the bases, and consequently hinder the DNA transcription and translation processes (26). It has also been reported that PRO can reverse the phenotypes of MDR bacteria (14).

Conclusion:

Our study demonstrated *in vitro* antimicrobial efficacies and time kill kinetics of amlodipine, thioridazine and promethazine against pathogenic clinical bacterial isolates, which indicates that these non-antibiotic drugs may be useful therapeutic alternatives in the bid to reduce the burden of infectious diseases associated with antibiotic resistant pathogens. Consequent upon these findings, *in vivo* antibacterial studies of these non-antibiotic drugs are required.

Authors' contributions:

AOJ and SGI designed the study, wrote the protocol and first draft of the manuscript. UAN and AMF wrote part of the manuscript and managed the analyses of the study. AOJ and OGO managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

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