JOURNAL OF MEDICINAL FOOD
J Med Food 7 (3) 2004, 327–333
© Mary Ann Liebert, Inc. and Korean Society of Food Science and Nutrition

In Vitro Antimicrobial Properties of Aqueous Garlic Extract Against Multidrug-Resistant Bacteria and *Candida* Species from Nigeria

B.A. Iwalokun,[1] A. Ogunledun,[2] D.O. Ogbolu,[3] S.B. Bamiro,[4] and J. Jimi-Omojola[2]

[1]*Biochemistry Department, Lagos State University;* [4]*Department of Obstetric and Gynaecology, College of Medicine, University of Lagos, Lagos;* [2]*Medical Microbiology and Parasitology Department, College of Health Science, Olabisi Onabanjo University, Sagamu; and* [3]*Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan, Nigeria*

ABSTRACT The antimicrobial effects of aqueous garlic extract (AGE) against 133 multidrug-resistant gram-positive and gram-negative bacterial isolates, including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Shigella* spp., and *Proteus* spp., and against 10 *Candida* spp. were studied. Antibacterial activity of AGE by well-diffusion and macrobroth dilution method was characterized by inhibition zones of 20.2–22.7 mm for gram-positives and 19.8–24.5 mm for gram-negatives and minimum inhibitory concentration (MIC) ranges of 15.6–48.3 mg/mL and 22.9–37.2 mg/mL, respectively. With the exception of *P. aeruginosa,* the observed disparity in MIC values at 24 and 48 hours was not significant ($P > .05$) in these isolates. The anticandidal effect of AGE resulted in a growth inhibition zone of 27.4 ± 3.7 mm with no significant difference ($P > .05$) in MIC values at 24 and 48 hours, respectively. Minimum fungicidal concentrations were found to be 14.9 and 15.5 mg/mL, respectively, at these incubation periods. Further analysis revealed the antimicrobial efficacy of AGE to be dose and time dependent, producing five distinct time–kill profiles among the isolates tested. The results of this study support the use of garlic in health products and herbal remedies in Nigeria.

KEY WORDS: • ***antibacterial effect*** • ***anticandidal effect*** • ***garlic*** • ***minimum inhibitory concentration*** • ***minimum fungicidal concentration***

INTRODUCTION

The continuous spread of multidrug-resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide.[1] In addition to increasing the cost of drug regimens, this scenario has paved way for the re-emergence of previously controlled diseases and has contributed substantially to the high frequency of opportunistic and chronic infection cases in developing countries.[2,3] The slow pace of newer antibiotic development coupled with the availability of fewer antifungal agents with fungicidal actions centered on inhibition of ergosterol synthesis has provided the need to explore nature in search of phytotherapeutic agents with novel targets and mode of actions. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections.[4,5] Garlic, a member of the Allium family (Liliaceae), has been used traditionally for ages to treat a wide array of diseases, namely, respiratory infections, ulcers, diarrhea, and skin infections, to mention just a few.[6] Reuter *et al.*[7] reported garlic as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and cardiovascular-protecting effects. This broad spectrum of activity has been attributed to the over 100 phytotherapeutic sulfur compounds present in varying concentrations in garlic. They include allicin and thiosulfinates, which are formed by crushing-induced metabolic action of the enzyme allicinase (a cysteine sulfoxide lyase) on the odorless amino acid allicin.[8] Variations in composition of garlic and genetic disparity among bacteria and fungi of the same or different species have been found responsible for the few inconsistencies in the antibacterial and antifungal properties of garlic extract,[9,10] necessitating the need for local antimicrobial testing of garlic. In Nigeria, most of the little work that has been done on garlic has provided the scientific basis for trypanocidal action of the plant,[11] which is not commensurate with the accelerated trend in garlic health products and herbal remedies in the country.

Manuscript received 19 October 2003. Revision accepted 13 February 2004.

Address reprint requests to: B.A. Iwalokun, Biochemistry Department, Lagos State University, PMB 1087, Apapa, Lagos, Nigeria, E-mail: bamwal@yahoo.com

Thus, there exists a wide gap in knowledge concerning antibacterial and antifungal profiles of this plant. As a prelude, an *in vitro* understanding of the antibacterial and antifungal properties of garlic against newer and re-emergent Nigerian isolates may broaden understanding of its spectrum of activity and provides scientific justification for use in therapeutic strategies against infections in the country.

The present study has investigated the antibacterial and antifungal activities of an aqueous garlic extract (AGE) against local multidrug-resistant bacteria and *Candida* spp.

MATERIALS AND METHODS

Garlic and aqueous extract preparation

Fresh bulbs of garlic (*Allium sativum* L.) were purchased from local markets in Ibadan, Nigeria. The cloves were separated and peeled to obtain the edible portion. Fifty grams of the edible portion was chopped and homogenized in 100 mL of autoclaved water in a Waring blender. The homogenate was then filtered by passage through a 25-μm-pore-size filter (Millipore, St. Quentin, France) to give a crude aqueous extract of 500 mg of garlic/mL. This was collected in a sterile vial and stored at 4°C until used.

Bacterial isolates

A total of 133 gram-positive and gram-negative bacterial isolates species were selected for study. The isolates were obtained from the Microbiology Department of University College Hospital, Ibadan, Nigeria and the Genetics Division of the Nigerian Institute of Medical Research. Bacteriologically, the isolates were identified as *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Haemophilus influenzae, Proteus mirabilis, Streptococcus pneumoniae,* and *Streptococcus pyogenes* using standard procedures.[12]

All the isolates were clinical strains recovered from patients with burns, diarrhea, bacteremia, bronchopulmonary disorder, and urinary tract infections. Antibiogram profiles of the organisms by the disk diffusion method revealed resistance to at least three antibiotics based on National Committee for Clinical Laboratory Standards guidelines[13] (Table 1). *H. influenzae, S. pneumoniae,* and *S. pyogenes* isolates were maintained on Robertson cooked meat medium (Oxoid, Basingstoke, UK), while the other isolates were maintained on tryptic soy agar slants at 4°C prior to use.

Candida spp.

A total of 10 *Candida* spp. recovered from vaginal swabs, urine, and blood samples were selected for study. The isolates were maintained on Yeast extract Potato Dextrose (YPD) agar containing 2% (vol/vol) glucose, 2% (wt/vol) Bacto peptone (Difco, Detroit, MI), 1% yeast extract (Difco), and 1% Bacto agar (Difco) at 4°C prior to use.

Antibacterial testing

The growth response of the selected bacterial and yeast isolates to crude garlic extract (500 mg/mL) was determined by the agar-well diffusion technique. Each bacterial isolate was inoculated into tryptic soy broth (Difco) and grown overnight at 37°C. The cells were then pelleted by centrifugation (3,000 rpm for 5 minutes), washed three times with phosphate-buffered saline (pH 7.5), and adjusted to an inoculum of 10^8 colony-forming units (cfu)/mL (0.5 McFarland standard) by the same buffer. In the case of *Candida* spp., cultures were grown overnight in liquid YPD

TABLE 1. BACTERIAL ISOLATES AND ANTIBIOTIC RESISTANCE PROFILES

Isolate[a]	*Number of isolates tested*	*Antibiotic resistance*[b]
S. typhi[a]	10	Cot, Col, Tet, Str, Chl, Amp
Shigella spp.	4	Cot, Col, Tet, Str, Chl, Amp, TMP-STX
S. aureus	28	Str, Amp, Pen, Amo, Aug
S. aureus	14	Str, Amp, Pen, Amo, Cef, Cro
Staphylococcus epidermidis	7	Str, Amp, Amo, Pen
S. aureus	1	Str, Amp, Amo, Aug, Pen, Met
E. coli	15	Cot, Col, Chl, Str, Tet
H. influenzae	10	Ery, Str, Cot, Amo, Amp, Tet
Proteus spp.	10	Amp, Tet, Cot, Col, Str, Cot
S. pneumoniae	15	Ery, Str, Col, Tet, Amo
S. pyogenes	10	Ery, Col, Tet, Chl, Amp, Amo
P. aeruginosa	18	Str, Ery, Tet, Kan, Col, Cot, Amo
P. aeruginosa	2	Str, Ery, Kan, Gen, Tet, Col, Aug

[a]Isolates were grouped based on their display of 100% resistance to antibiotics by disk diffusion assay following identification accrding to the methods of Cowan and Steel.[12]

[b]Str = streptomycin; Ery = erythromycin; Aug = augmentin; Cef = cefotaxime; Cro = ceftriaxone; Tet = tetracycline; Amp = ampicillin; Amo = amoxicillin; Kan = kanamycin; Gen = gentamicin; Chl = chloramphenicol; Col = colistin sulfate; Cot = cotrimoxazole; TMP-STX = trimethoprim–sulfamethoxazole.

medium at 30°C, and the inoculum was adjusted to 10^8 cfu/mL.

One hundred microliters of inoculum (10^7 cells) was inoculated into 20 mL of appropriate molten agar (42°C) and subsequently poured into petri dishes. One hundred microliters of crude garlic extract was then dispensed into 6-mm precut wells (four wells per plate), and plates were incubated at 37°C in the case of bacteria and at 30°C in the case of *Candida* isolates. Negative control plates for bacteria were prepared by dispensing 100 μL of freshly prepared 50 μg/mL (wt/vol) ciprofloxacin (Oxoid) in sterile distilled water into the wells. The negative YPD plates contained wells filled with 100 μL of 20 μg/mL (wt/vol) fluconazole (Neimeth, Lagos, Nigeria). Wells filled with 100 μL of phosphate-buffered saline served as positive control. Two ciprofloxacin-sensitive standard strains, *E. coli* ATCC25922 and *S. aureus* ATCC25923, were also inoculated in parallel with test organisms. The zones of inhibition observed were measured in millimeters after 24 hours for the bacterial isolates and 48 hours for the *Candida* isolates.

Minimum inhibitory concentration (MIC) determination

The MIC of the AGE against each of the tested isolates was determined by the macrobroth dilution method. AGE solutions at concentrations of 0–25 mg/mL by serial twofold serial dilutions and concentrations at 50, 45, 40, 35, and 30 mg/mL were prepared in tryptic soy broth in the case of bacteria and liquid YPD medium in the case of *Candida* spp. The tubes were then inoculated with 0.1 mL of cultures (10^7 cells). Uninoculated tubes containing growth medium or growth medium and extract were used as controls. The tubes were then incubated at appropriate temperatures for 24 and 48 hours. The MIC was defined as the lowest concentration of garlic extract that completely inhibited the growth of the organisms.

Minimum fungicidal concentration (MFC) determination

The MFC of the AGE was determined by plating 100-μL samples from each MIC assay tube with growth inhibition onto YPD agar medium. Plates were then incubated at 30°C for 24–48 hours. The MFC was defined as the lowest concentration of garlic extract that produced three or fewer colonies after incubation.[14]

Time–kill analysis

In vitro killing of AGE against the tested isolates was investigated according to the method of Yin *et al*.[15] A bacterial inoculum of 10^6 cfu/mL or a yeast inoculum at 10^4 cfu/mL was seeded into 10 mL of reaction medium containing tryptic soy broth or liquid YPD and garlic extract at 1–3× MIC for 24 hours at 37 or 30°C. A 100-μL aliquot was removed from the culture medium every 2 hours for 12 hours for the determination of cfu/mL by the plate count technique.[16] Viable counts were read after a 24-hour incubation. Garlic-deficient assays were used as controls. The limit of detection was 20 cfu/mL.

TABLE 2. PRELIMINARY ANTIMICROBIAL TESTING OF THE CRUDE AGE

	Inhibition zone diameter		
Isolate[a]	*AGE*	*Standard drugs*	*PBS (positive control)*
Gram-positive bacteria			
S. aureus (28)	21.6 ± 2.5	27.3 ± 1.6	G
S. aureus (14)[b]	21.8 ± 2.7	26.7 ± 1.9	G
S. aureus (2)[c]	22.5 ± 2.1	29.0 ± 1.4	G
S. epidermidis (6)	22.7 ± 1.6	28.3 ± 1.5	G
S. pneumoniae (15)	20.2 ± 1.7	19.2 ± 2.3	G
S. pyrogenes (10)	21.6 ± 1.6	24.5 ± 2.2	G
Gram-negative bacteria			
S. typhi (10)	23.8 ± 1.5	28.4 ± 1.3	G
Shigella spp. (4)	24.5 ± 3.0	29.3 ± 1.2	G
E. coli (15)	23.7 ± 2.4	28.7 ± 1.3	G
Proteus spp. (10)	23.2 ± 1.9	21.5 ± 1.4	G
P. aeruginosa (9)[d]	19.8 ± 1.2	21.9 ± 1.7	G
H. influenzae (10)	20.7 ± 2.9	24.6 ± 1.3	G
Fungal isolates			
Candida spp. (10)	27.4 ± 3.7	29.8 ± 2.6	G

The diameters of zone of inhibition were expressed as mean ± standard deviation values (mm). G indicates bacterial growth around the phosphate-buffered saline (PBS) control wells. Standard drugs used were ciprofloxacin (5 μg per well) and fluconazole (2 μg per well).

[a]Numbers in parentheses represent the number of isolates tested.

[b]The *S. aureus* strains also showed 100% resistance to cefuroxime, ceftriaxone, and cefotaxime.

[c]The strains were beta-lactamase producers and also showed 100% resistance to penicillin (1 unit) and augmentin.

[d]All the tested *P. aeruginosa* strains showed sensitivity to ciprofloxacin, while nine of the isolates were sensitive to AGE.

TABLE 3. MIC DETERMINATIONS OF AGE AGAINST MULTIDRUG-RESISTANT BACTERIA AND *CANDIDA* SPP.

Isolate	*MIC (mg/mL) in AGE assay*		t	P
	24 hours	*48 hours*		
Gram-positive bacteria				
S. aureus (28)	27.1 ± 4.2	28.1 ± 3.4	0.96	>0.05
S. aureus (14)[a]	24.6 ± 5.9	26.3 ± 5.1	0.78	>0.05
S. aureus (2)[b]	25.0 ± 0.0	27.5 ± 3.5	0.71	>0.05
S. epidermidis (6)	22.9 ± 5.1	25.8 ± 2.1	1.17	>0.05
S. pneumoniae (15)	30.3 ± 4.4	32.7 ± 5.9	1.21	>0.05
S. pyogenes (10)[c]	33.0 ± 5.4	37.2 ± 7.5	1.30	>0.05
H. influenzae (10)	30.5 ± 5.5	34.5 ± 4.9	1.62	>0.05
Gram-negative bacteria				
S. typhi (10)	21.8 ± 6.6	23.5 ± 6.1	0.57	>0.05
Shigella spp. (4)	15.6 ± 6.3	23.1 ± 7.5	1.33	>0.05
E. coli (15)	20.8 ± 6.1	22.5 ± 5.2	0.79	>0.05
Proteus spp. (10)	23.0 ± 5.7	25.3 ± 5.1	0.90	>0.05
P. aeruginosa (14)[d]	32.5 ± 5.8	48.3 ± 4.1	36.98	<0.05
Fungal isolates				
Candida spp. (10)	14.9 ± 7.0	12.5 ± 5.1	0.21	>0.05

MICs were expressed as mean ± standard deviation values. t = Student's t test; P = probability at 5% significance level.
[a]The *S. aureus* strains also showed 100% resistance to cefuroxime, ceftriaxone, and cefotaxime.
[b]The strains were beta-lacamase producers and also showed 100% resistance to penicillin (1 unit) and augmentin.
[c]In two of the 10 *S. pyogenes* isolates, MICs >50 mg/mL were observed after 48 hours of incubation in trypticase soy broth.
[d]In nine of the 14 *P. aeruginosa* isolates, MICs >50 mg/mL were observed after 48 hours of incubation in trypticase soy broth.

Statistical analysis

All the parameters measured were expressed as means ± standard deviation. The difference between mean values was analyzed by Student's t test at the 5% significance level. $P < .05$ was considered to be significant, and $P > .05$ was non-significant.

RESULTS

The present study has investigated the antimicrobial activity of AGE against local bacterial and *Candida* isolates in Nigeria. Table 2 summarizes the result of the preliminary investigation of crude AGE at 500 mg/mL. The extract exhibited growth inhibition of both gram-positive and gram-negative bacteria on tryptic soy agar with inhibition zone diameter of 20.2–22.7 ± 1.7–1.6 mm for gram-positive bacteria and 19.8–24.5 ± 1.2–3.0 mm for gram-negative bacteria. Sensitivity of these isolates was further confirmed in ciprofloxacin plates (inhibition zone diameter = 19.2–29.3 ± 2.3–1.2 mm). Growth inhibition of *Candida* isolates on YPD was also observed resulting in a zone of inhibition of 27.4 ± 3.7 mm due to AGE and 29.8 ± 2.6 mm due to fluconazole.

Further antibacterial activity testing of AGE by the macrobroth dilution method also revealed growth inhibition in liquid medium with MICs of 15.6–48.3 mg/mL and 22.9–37.2 mg/mL for gram-negatives and gram-positives, respectively. With the exception of *P. aeruginosa,* the observed disparity in MIC values at 24 and 48 hours was not significant ($P > .05$) in these isolates (Table 3).

Further evaluation of the anticandidal activity of AGE was characterized by MICs of 11.9 ± 5.5 and 12.5 ± 5.1 mg/mL (Table 3) and MFCs of 14.9 ± 7.0 and 15.5 ± 6.4 mg/mL (Table 4) at 24 and 48 hours, respectively.

AGE bacterial growth inhibition and anticandidal efficacy were observed to be dose and time dependent, producing five distinct time–kill profiles for Entobacteriaceae, *Staphylococcus, Streptococcus, Haemophilus,* and *Candida* isolates tested (Fig. 1).

TABLE 4. MFC DETERMINATION OF AGE AGAINST *CANDIDA* SPP.

Fungal isolate	*MFC (mg/mL) in AGE assay*		t	P
	24 hours	*48 hours*		
Candida spp. (10)	14.9 ± 7.0	15.5 ± 6.4	0.19	>0.05

MFCs were expressed as mean ± standard deviation values. t = Student's t test; P = probability at 5% significance level.

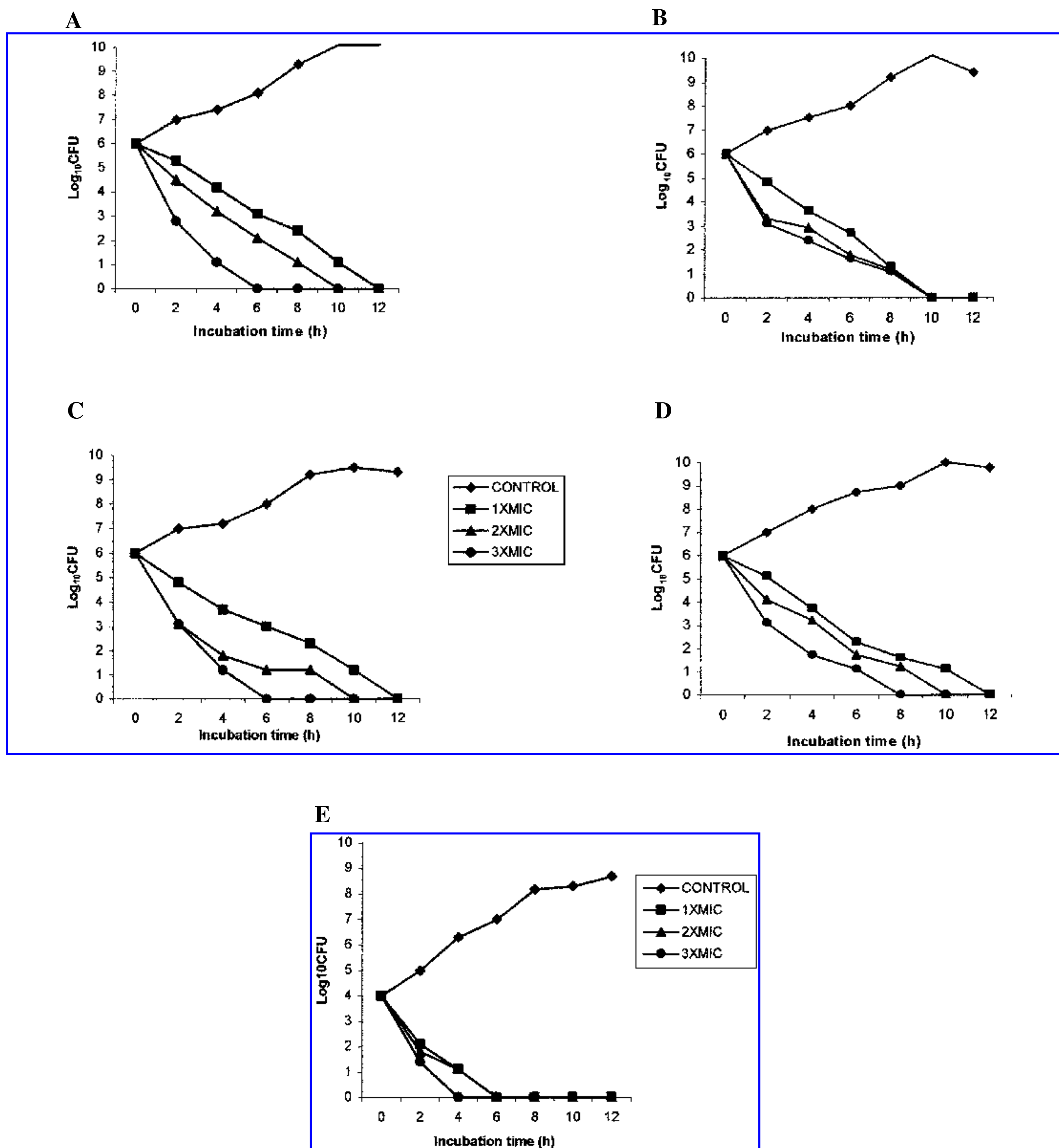

FIG. 1. Time–kill analysis of AGE at various MICs against multidrug-resistant (**A**) Enterobacteriaceae (n = 30), (**B**) *Staphylococcus* spp. (n = 21), (**C**) *Streptococcus* spp. (n = 20), (**D**) *H. influenzae* (n = 10), and (**E**) *Candida* spp. (n = 10) within 12 hours.

DISCUSSION

Garlic has been known for ages to have anti-infective properties against a wide range of microorganisms.[17] The present study has further demonstrated the antimicrobial potency of AGE against local multidrug-resistant bacteria and *Candida* isolates from Nigeria. The observed zones of growth inhibition on agar of gram-positive and gram-nega-

tive bacteria and *Candida* isolates were comparable to those elicited by ciprofloxacin and fluconazole, showing that the isolates exhibited susceptibility. This indicates that AGE has a broad spectrum of antimicrobial activity and a wide therapeutic window. The isolates tested in this study are responsible for many diseases in Nigeria, including bacteria meningitis, maxillary sinusitis, and otolaryngological diseases by *S. pneumoniae, S. pyogenes,* and *H. influenzae,*[18,19] bronchopulmonary disorders and chronic otitis media by *P. aeruginosa,*[20,21] candidiasis and vaginitis by *Candida albicans,*[22] nosocomial infections and bacteremia due to multidrug-resistant staphylococcal infections,[23,24] and diarrheal diseases caused by *E. coli,*[25] *Shigella* spp.,[26] and *S. typhi*.[27] The sensitivity of these isolates to AGE also implies that the intrinsic biosubstances in this extract are naive to the various drug resistance factors of the isolates, which include beta-lactamase expression, increased pyrrolidonylarylamidase activity, aminoglycoside-modifying enzymes, and altered ribosomal binding.[28,29] Meanwhile, the antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis.[30,31]

Several studies, including those of Rees *et al*.[32] and Kumar and Sharma,[33] had previously demonstrated the antibacterial potency of AGE against enteropathogens such as *Vibrio parahaemolyticus*, *E. coli*, *Klebsiella* spp., *Proteus* spp., and *S. aureus*[7] and anticandidal effects against *Candida* spp.[34] In spite of geographical variation, the MICs of AGE for our isolates are consistent with those of Sivam[35] but are relatively lower than values obtained by Ross *et al*.[36] This antimicrobial potency disparity of garlic has been attributed to the different concentrations of individually and synergistically active biosubstances in garlic preparations coupled with their interactions with sulfhydryl agents in culture media. This phenomenon has been used to explain the stronger antimicrobial effect of allicin than garlic oil disulfides.[37] Meanwhile, allicin and other diallylsulfide compounds have been found at different concentrations in AGE determined by age and method of extract preparation.[38]

The MFCs of AGE reported here are in consonance with previous anticandidal studies on pathogenic and emerging drug-resistant *Candida* species: *C. albicans, Candida glabrata,* and *Candida krusei*.[14,39] It can be said that the concentration at which AGE showed growth inhibition is also fungicidal to our isolates as it displayed comparable MIC and MFC values. We monitored growth inhibition at 48 hours of incubation to investigate the possibility of cfu rebound due to extract instability or volatilization of bioactive agents in garlic, culminating in significantly increased MICs. This expectation was only observed in *P. aeruginosa* isolates. The susceptibility response observed of some of the *P. aeruginosa* isolates aligns with the finding of Tsao and Yin[40] but at variance with the work of Kivanc and Kunduhoglu.[9] The significantly increased MICs at 48 hours postinoculation may be the consequence of cfu rebound and bacteriostatic effect of AGE on these strains. The reliability of the clinical interpretation of this observation may undoubtedly require further tolerability testing of AGE in humans as a prelude to understanding garlic-induced plasma resistance to infections.

The observation that AGE elicited its antimicrobial potency in a dose- and time-dependent manner producing distinct time–kill profiles suggests variations in the growth inhibitory responses of the tested isolates to garlic. Similar responses have been observed in antibiotic-resistant *E. coli, Enterobacter cloacae,* and *Citrobacter freundi*.[15] However, the uniqueness of time–kill profiles of the gram-positives and gram-negatives in this study may be connected with their structurally different cell wall barriers. The lipid composition of cell wall has been found to have an influence on the permeability of hydrophobic and volatile bioactive substances in garlic.[41] The eukaryotic nature and ergosterol availability in *Candida* cell wall may also be crucial to the observed time–kill kinetics of AGE against our isolates.

In conclusion, the results of this study have provided scientific justification for the use of garlic extract in health products and herbal remedies against multidrug-resistant bacterial infections and candidiasis in Nigeria. Therefore, complementary and alternative medicine practices with plant extracts including garlic as a means of decreasing the burden of drug resistance and reducing the cost of management of diseases would be of clinical and public health importance in this country.

ACKNOWLEDGMENTS

The authors wish to thank the technical staff of the Genetics Department, Nigerian Institute of Medical Research, and the Microbiology Department of the University College Hospital, Nigeria for the supply of standard strains and isolates used in this study.

REFERENCES

1. Sanders CC, Sanders WE Jr: β-Lactamase resistance in gram-negative bacteria: global trends and clinical impact. *Clin Infect Dis* 1992;15:824–839.
2. Fernandez Guerrero ML, Ramos JM, Marrero J, Cuenca M, Fernandez Roblas R, de Gorgolas M: Bacteremic pneumococcal infections in immunocompromised patients without AIDS: the impact of beta-lactam resistance on mortality. *Int J Infect Dis* 2003;7:46–52.
3. Ako-Nai AK, Ikem IC, Aziba A, Ajayi AA, Onipede OA: Bacteriological examination of chronic osteomyelitis cases in Ile-Ife, SouthWestern Nigeria. *Afr J Clin Exp Microbiol* 2003;4:41–51.
4. Vijaya K, Ananthan S: Microbiological screening of Indian medicinal plants with special reference to enteropathogens. *J Altern Complement Med* 1997;3:13–20.
5. Dilhuydy JM: Patients' attraction to complementary and alternative medicine (CAM): a reality which physicians can neither ignore nor deny. *Bull Cancer* 2003;90:623–628.
6. Fenwick GR, Hanley AB: The genus *Allium*—part 3. Medicinal effects. *CRC Crit Rev Food Sci Nutr* 1985;23:1–73.
7. Reuter HD, Koch HP, Lawson LD: Therapeutic effects and applications of garlic and its preparations. In: *Garlic. The Science*

and Therapeutic Application of Allium sativum *L and Related Species* (Koch HP, Lawson LD, eds.), Williams and Wilkins, Baltimore, 1996, pp. 135–512.

8. Lawson LD, Wood SG, Hughes BG: HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. *Planta Med* 1991;57:263–270.
9. Kivanc M, Kunduhoglu B: Antimicrobial activity of fresh plant juice on the growth of bacteria and yeasts. *J Qafqaz University* 1997;1:27–35.
10. Lawson LD: Garlic: a review of its medicinal effects and indicated active compounds. In: *ACS Symposium Series 691: Phytomedicines of Europe: Chemistry and Biological Activity* (Lawson LD, Bauer R, eds.), American Chemical Society, Washington, DC, 1998, pp. 176–209.
11. Okochi VI, Kazeem AA, Gbenle GO, Fagbenro-Beyioku AF, Dare A, Arukwe U: Effect of *Allium sativum* (garlic) water extract on *Trypanosoma brucei* infection in laboratory rat. *Biokemistri* 2000;11:9–15.
12. Cowan ST, Steel KJ: *Manual for the Identification of Medical Bacteria,* 2nd ed., Cambridge University Press, Cambridge, UK, 1974.
13. National Committee for Clinical Laboratory Standards: *Performance for Antimicrobial Susceptibility Testing: Sixth International Supplement,* NCCLS document M100–s6, National Committee for Clinical Laboratory Standards, Wayne, PA, 1995.
14. Onyewu C, Blankenship JR, Del Poeta M, Heitman J: Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against *Candida albicans, Candida glabrata,* and *Candida krusei. Antimicrob Agents Chemother* 2003;47:956–964.
15. Yin MC, Chang HC, Tsao SM: Inhibitory effects of aqueous garlic extract, garlic oil and four diallylsulphides against four enteric pathogens. *J Food Drug Anal* 2002;10:120–126.
16. Cruishank R, Duguid JP, Marmion BP, Swain RHA: *Medical Microbiology. The Practice of Medical Microbiology,* 12th ed., Churchill Livingstone, London, 1975.
17. Oliver B: Medicinal plants in Nigeria. *Nigerian Coll Arts Sci Technol* 1960;1:1–137.
18. Ijaduola GTA, Anyiwo CE: Microbiological study of chronic maxillary sinusitis in Nigerian adults. *Nigerian J Microbiol* 1984;4:49–54.
19. Bhatia PC, Varughese R: Pattern of otolaryngological disease in Jos community. *Nigerian Med J* 1987;17:67–93.
20. Iroegbu CU, Njoku-Obi AN: Bacterial agents associated with bronchopulmonary disorders in eastern Nigeria. *Arch Roum Pathol Exp Microbiol* 1990;49:43–50.
21. Adeyeba OA, Adeoye MO, Adesiji YO: Bacteriological and parasitic assessment of vaginitis in pregnant women in Iseyin, Oyo State, Nigeria. *Afr J Clin Exp Microbiol* 2003;4:116–126.
22. Obi CL, Enweani IB, Giwa JO: Bacterial agents causing chronic suppurative otitis media. *East Afr Med J* 1995;72:370–372.
23. Ako-Nai AK, Adejuyigbe EA, Ajayi FM, Onipede AO: The bacteriology of neonatal septicaemia in Ile-Ife, Nigeria. *J Trop Pediatr* 1999;45:146–151.
24. Okesola AO, Oni AA, Bakare RA: Nosocomial infections: methicilllin resistant *Staphylococcus aureus* in wound infection in Ibadan, Nigeria. *Afr J Med Med Sci* 1999;28:55–57.
25. Olukoya DK, Daini O, Alabi SA, Coker AO, Odugbemi T, Akinrimisi EO: Antimicrobial resistance patterns and plasmids of enteropathogenic *Escherichia coli* isolated in Nigeria. *Eur J Epidemiol* 1988;4:306–309.
26. Iwalokun BA, Gbenle GO, Smith SI, Ogunledun A, Akinsinde KA, Omonigbehin EA: Epidemiology of shigellosis in Lagos, Nigeria: trends in antimicrobial resistance. *J Health Popul Nutr* 2001;19:183–190.
27. Akinyemi KL, Olukoya DK, Oyefolu AO, Amorighoye EP, Omonigbehin EO: Prevalence of multiple drug resistance *Salmonella typhi* among clinically diagnosed typhoid fever patients in Lagos, Nigeria. *Z Naturforsch* 2000;55:489–493.
28. Cercenado E, Vicente MF, Diaz MD, Sanchez-Carrillo C, Sanchez-Rubiales M: Characterization of clinical isolates of beta-lactamase-negative, highly ampicillin-resistant *Enterococcus faecialis. Antimicrob Agents Chemother* 1996;40:2420–2422.
29. Paparaskevas J, Vatopoulos A, Tassios PT, Avliami A, Legakis NJ, Kalapothaki V: Diversity among high-level aminoglycoside-resistant enterococci. *J Antimicrob Chemother* 2000;45:277–283.
30. Dewitt JC, Notermanns S, Gorin N, Kampelmacher EH: Effect of garlic oil or onion oil on toxin production by *Clostridium botulinum* in meat slurry. *J Food Protect* 1979;42:222–224.
31. Willis ED: Enzyme inhibition by allicin, the active principle of garlic. *J Biochem (Tokyo)* 1956;63:514–520.
32. Rees LP, Minney SF, Plumer NT, Slater JH, Skyme DA: A quantitative assessment of antimicrobial activity of garlic (*Allium sativum*). *World J Microbiol Biotechnol* 1993;9:303–307.
33. Kumar A, Sharma VD: Inhibitory effect of garlic (*Allium sativum* Linn) on enterotoxigenic *Escherichia coli. Indian J Med* 1982;76:66–70.
34. Adetunmbi M, Taylor GT, Lau BHS: *Allium sativum* (garlic) inhibits lipid synthesis by *Candida albicans. Antimicrob Agents Chemother* 1986;30:499–501.
35. Sivam GP: Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J Nutr* 2001;131(Suppl):1106S–1108S.
36. Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ: Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparison with garlic sulfides and garlic powder. *Appl Environ Microbiol* 2001;67: 475–480.
37. O'Gara EA, Hill DJ, Maslin DJ: Activities of garlic oil, garlic powder and their diallyl constituents against *Helicobacter pylori*. *Appl Environ Microbiol* 2000;66:2269–2273.
38. Lawson DL: The composition and chemistry of garlic cloves and processed garlic. In: *Garlic: The Science and Therapeutic Applications of* Allium sativum *L and Related Species,* 2nd ed. (Koch HP, Lawson DL, eds.), Williams and Wilkins, Baltimore, 1996, pp. 37–107.
39. Ghannoum MA: Studies on the anticandidal mode of action of *Allium sativum* (garlic). *J Gen Microbiol* 1988;134:2917–2924.
40. Tsao SM, Yin MC: *In vitro* activity of garlic oil and four sulphides against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae. J Antimicrob Chemother* 2001;47: 665–670.
41. White TC, Marr KA, Bowden RA: Clinical, cellular and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev* 1998;11:382–402.

This article has been cited by:

1. Ali Aydin , Kamil Bostan , Mehmet Emin Erkan , Bariş Bingöl . 2007. The Antimicrobial Effects of Chopped Garlic in Ground Beef and Raw Meatball (Çiğ Köfte). *Journal of Medicinal Food* **10**:1, 203-207. [Abstract] [PDF] [PDF Plus]
2. Noori S. Al-Waili , Khelod Y. Saloom , M. Akmal , Thia N. Al-Waili , Ali N. Al-Waili , Hamza Al-Waili , Amjed Ali , Karem Al-Sahlani . 2007. Effects of Heating, Storage, and Ultraviolet Exposure on Antimicrobial Activity of Garlic Juice. *Journal of Medicinal Food* **10**:1, 208-212. [Abstract] [PDF] [PDF Plus]
3. Hyeon-Hee Yu, Kang-Ju Kim, Jeong-Dan Cha, Hae-Kyoung Kim, Young-Eun Lee, Na-Young Choi, Yong-Ouk You. 2005. Antimicrobial Activity of Berberine Alone and in Combination with Ampicillin or Oxacillin Against Methicillin-Resistant Staphylococcus aureus . *Journal of Medicinal Food* **8**:4, 454-461. [Abstract] [PDF] [PDF Plus]