A COMPARATIVE ASSESSMENT OF THE ANTIMICROBIAL EFFECTS OF GARLIC (*ALLIUM SATIVUM*) AND ANTIBIOTICS ON DIARRHEAGENIC ORGANISMS

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Abstract. Antimicrobial sensitivity tests were carried out on *Escherichia coli, Shigella* sp, *Salmonella* sp, and *Proteus mirabilis* using standard procedures. Significant differences ($p<0.01$) were seen in the effect of the antimicrobial agents (garlic, ciprofloxacin and ampicillin), and in the sensitivities of the microbial species ($p<0.01$) to the antimicrobial agents were observed. The gram-negative diarrheagenic pathogens from the stool samples were highly sensitive to garlic, while ciprofloxacin (CPX) was most effective against *E. coli.* The differences were inferred to result from genetic differences among the organisms and differences in the modes of action of the antibiotics. No isolates were resistant to garlic, making it a promising antimicrobial agent. It appears that antibiotics that interfere with DNA and RNA syntheses, such as garlic does, could constitute an effective partner in the synergic effect of garlic currently being investigated worldwide.

INTRODUCTION

Garlic is a perennial bulb-forming plant that belongs to the genus *Allium* in the family Liliaceae, along with leeks (*A. porrum*), onions (*A. cepa*) and chives (*A. schoenoprasum*). For several centuries, garlic has been known to possess dietary and medicinal properties (Ross *et al*, 2001). Several studies have proved that garlic has antimicrobial effects (Rees *et al,* 1993; Reuter *et al*, 1996; Lawson, 1998; Martin and Ernst, 2003). It inhibits the growth of both gram-negative and gram-positive bacteria, the same as molds and yeasts (Pai and Platt, 1992; Ross *et al*, 2001). Increased consumption of *Allium* vegetables decreases the risk of gastric cancer possibly because of the effect of garlic on *Helicobacter pylori*, as this organism is associated with gastric cancer (Cellini *et al,* 1996).

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The antimicrobial activity of garlic has been attributed to the presence of thiosulfinates (*eg*, allicin) whose removal completely renders garlic ineffective against microorganisms (Hughes and Lawson, 1991). Allicin is obtained by crushing or cutting garlic cloves. The ordorless amino acid, alliin, present in the garlic cloves, is metabolized by the enzyme allinase (a cysteine sulfoxide lyase) to allicin and other thiosulfinates, which besides their antimicrobial effects, produce the characteristic odor of garlic (Block, 1985). Allicin acts by totally inhibiting RNA synthesis and partially inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin (Feldberg *et al,* 1988). Bacterial susceptibility to garlic may also depend on structural differences of the bacterial strains. The cell wall of gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipid, whereas gram-positive bacteria contain 35-60% polysaccharides and only 0-2% lipid (Carpenter, 1968). Sivam (1998) reported that the cell membrane of *Escherichia coli* contains 20% lipid, whereas that of *Staphylococcus aureus* contains 2% lipid. The polysaccharide and lipid contents of the cell wall have an effect on the permeability of allicin and other garlic constituents; this may be responsible for the difference in susceptibility to garlic between gram-negative *Helicobacter pylori* and gram-positive *Staphylococcus aureus* (Cellini *et al*, 1996; Sivam *et al,* 1997).

Because of multiple uses of antimicrobial agents in medicine, production of food animals and crop protection, existing antimicrobial agents have declined in effectivity due to resistance of organisms to those agents. This is particularly evident in *Staphyloccus aureus* (Parker, 1978; Itah, 1999) and enteropathogenic bacteria, *eg*, *Shigella* and *Salmonella* species (Eja *et al,* 2006). Because of the magnitude of the problem of drug resistance, some researchers have chosen to develop alternative strategies (Sivam *et al,* 1997). Sivam *et al* (1997) notes that garlic has broad spectrum activity, and is known to act synergistically with antibiotics. No resistance has been reported but more clinical studies need to be done to assess the use of an antibiotic/garlic combination for bacteria that are difficult to eradicate.

In this study the aim was to carry out a comparative assessment of the antimicrobial effects of garlic and two known broadspectrum antibiotics whose mechanisms of action are known to be similar to that of garlic. Amplicillin was chosen as a comparison in this study because, similar to garlic which is known to inhibit cell wall synthesis, it inhibits transpeptidation enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall and also activates lytic enzymes (Prescott *et al*, 2005). Ciprofloxacin was the second drug chosen for comparison, because it inhibits bacterial DNA gyrase, and thus interferes with DNA transcription and other activities involving DNA (Prescott *et al,* 2005) similar to the action of garlic. This is part of the search for suitable antibiotics for synergism with garlic, or garlic alone against resistant enteropathogens.

MATERIALS AND METHODS

Sources of test organisms and garlic

The microorganisms used for this study were isolated from 22 stool samples from the University of Calabar Teaching Hospital (UCTH) and the General Hospital, Calabar (GHC) in southeastern Nigeria. The samples were collected and transported to the laboratory for analysis using prescribed procedures (Cheesbrough, 1991). The garlic used was bought from Watt Market, Calabar.

Microbiological analysis of samples

Samples were inoculated on tryptone soya agar (Oxoid, England), a general purpose agar that can support the growth of both aerobes and anaerobes when supplemented with 1% (W/V) cysteine hydrochloride (BDH Chemicals, UK) (APHA, 1998), MacConkey agar for the isolation of coli-aerogenes-like enteric organisms (Itah and Ben, 2004), and incubated at 37ºC for 24 hours. Primary isolates were repeatedly subcultured on fresh media using plate streaking techniques to obtain pure cultures. The purified isolates were stored in agar slant at 4ºC for later characterization and identification.

Lindquist's bacterial identification scheme (Lindquist, 1999) adopted from Cowan (1974) was used for the characterization and identification of the isolates. In this scheme, some heterotrophic bacterial genera were sorted out based on various primary tests such as gram reaction and shape of bacteria, cross-matched with the results of glucose fermentation tests, catalase and oxidase reactions, and other tests, such as a motility test, possession of endospores, aerobic and anaerobic growth.

Suspected *Escherichia* isolates were differentiated from *Klebsiella* by inoculating the isolates in lactose peptone water and incubating at 44ºC. Gas production confirmed *E. coli* which was further confirmed by indole Methyl Red Voges Proskauer (IMVIC) tests, since *Enterobacter aerogenes* has many characteristics in common with *E. coli.*

Preparation of garlic (*Allium sativum*) extract

Garlic cloves were peeled, cut into pieces and put in a juice extractor which was pressed to squeeze out the raw garlic extract. The raw extract (10.5 ml) weighing 1,120 mg/ml, was put in a sterile container and stored in the refrigerator at 4ºC for later use.

Testing for the antibacterial effects of raw garlic extract

A disc diffusion technique using the Kirby-Bauer method (Stokes and Ridgway, 1980; Prescott *et al,* 2005) was applied in testing pure cultures of the isolates for their antimicrobial sensitivities.

The sensitivity discs used for the garlic testing were punched from Whatman No.1 filter paper. The sterile discs 5 mm in diameter were impregnated with raw extract and dried in a hot air oven at 60ºC for 5 minutes (Onyeagba *et al,* 2004).

Agar plates (5 plates for each test organism per antimicrobial agent) were inoculated with 0.1 ml broth culture of test organisms and spread with a glass rod shaped like a hockey stick, and incubated at 37ºC for 24 hours. The antibiotics, ampicillin (AMP) and ciprofloxacin (CPX), were used as controls for comparison with the raw extract. The plates were observed for zones of inhibition after incubation followed by calculation of the mean zones of inhibition (mm).

Statistical analysis

Differences, if any, between garlic and antibiotics were determined using statistical analysis (ANOVA) (Bailey, 1981; Miller and Miller, 1986).

RESULTS

Bacterial isolates from the stool samples were principally *Escherichia coli, Shigella* sp, *Salmonella* sp and *Proteus mirabilis*.

The sensitivity patterns of the bacterial isolates from stool samples are shown in Fig 1, which shows the zones of inhibition of bacterial growth by garlic, CPX and AMP. *E. coli* was most sensitive to ciprofloxacin. The sensitivities of *E. coli* and *Salmonella* sp to garlic were the same and appreciable. Ampicillin was least effective against all organisms. The highest sensitivity to garlic was exhibited by *Shigella* sp while *P. mirabilis* was least sensitive

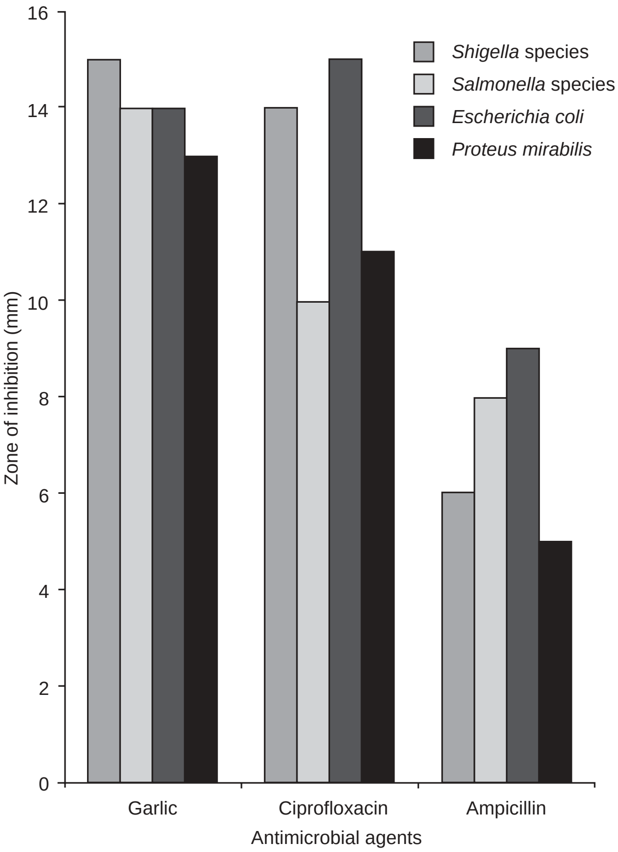

Fig 1–Antimicrobial sensitivity of microorganisms from stool samples, to garlic, ciproflaxacin and ampicillin.

to garlic. Garlic and CPX exhibited comparable effects on stool isolates which were mostly gram-negative diarrheagenic organisms as shown in Fig 1.

Analysis of variance (ANOVA) showed a significant difference ($p < 0.01$) in efficacy between antimicrobial agents (Garlic, CPX and AMP), and between the microbial species with respect to their sensitivities to the antimicrobial agents. The test of Least Significant Difference (LSD) showed that garlic and CPX were each significantly different from AMP at 95, 99 and 99.9% levels of confidence. There was, however, no significant difference between garlic and CPX, indicating that garlic and CPX had similar levels of antimicrobial effects, while AMP was less effective.

DISCUSSION

In this study, garlic was observed to have an antimicrobial effect on all the bacterial isolates, similar to CPX and AMP, broad spectrum antibiotics. Garlic exhibited broad spectrum activity against gram-positive and gram-negative bacteria (Farbman *et al,* 1993; Ross *et al,* 2001).

Both garlic and CPX showed exceptional antimicrobial activity against the bacterial species isolated in this study, which were *Escherichia coli, Shigella* sp, *Salmonella* sp and *Proteus mirabilis*. Garlic and CPX had similar levels of antimicrobial activity against diarrheagenic organisms (Fig 1). This was attributed to similarity in the mechanism of action of garlic and CPX. Allicin, the active ingredient of garlic (Hughes and Lawson, 1991), acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Feldberg *et al,* 1988). Similarly, CPX inhibits bacterial DNA gyrase, and thus interferes with DNA transcription and other activities involving DNA (Prescott *et al*, 2005). There was also some similarity in the modes of action of garlic and AMP, as both inhibit cell wall synthesis (Prescott *et al,* 2005). For all bacterial isolates, garlic showed greater antimicrobial activity than AMP. This is probably due to resistance to AMP arising from long-term and indiscriminate use of AMP, and the fact that antimicrobial resistance is not known to occur in garlic. Garlic is active even against organisms that have become resistant to antibiotics (Sivam, 1998).

There was a significant difference ($p< 0.01$) in effectivity between garlic, CPX and AMP, which could have arisen as a result of genetic differences in the sensitivities of the isolates to the antimicrobial agents and differences in the modes of action of the antibiotics. The minimum inhibition zone of garlic to any of the isolates was 12.5 mm, found with *Proteus mirabilis,* whereas those of CPX and AMP were 8.8 and 4.5 mm, respectively. Garlic is the "drug" of choice in the treatment of infections due to diarrheagenic organisms. *H. pylori* sensitivity to garlic has also been observed (Cellini *et al*, 1996; Sivam *et al,* 1997). There is great concern about the worldwide increase in antibiotic resistance (Jonkers *et al,* 1999a), thus, the use of garlic for the treament of infections may be a solution to the drug resistance problem.

In this study gram-negative bacterial isolates were more sensitive to garlic. The cell wall structural nature of gram-negative enteric bacteria may be responsible for the observed susceptibility. For instance, the cell wall of gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipid, whereas that of gram-positive bacteria contains 35-60% polysaccharides and only 0-2% lipid (Carpenter, 1968). The cell membrane of *E. coli* has been reported to contain 20% lipid (Sivam, 1998). The polysaccharides and the lipid contents of the cell wall affect the permeability of allicin and other garlic constituents, and thus the observed susceptibility to garlic by the diarrheagenic organisms (Cellini *et al,* 1996; Sivam *et al,* 1997).

Enterotoxic *E. coli* strains and other pathogenic intestinal bacteria, which are diarrheagenic in humans and animals, have been reported to be easily inhibited by garlic (Caldwell and Danzer, 1988). The gastric environment, such as pH, temperature and dietary factors may influence antimicrobial activity (Jonkers *et al,* 1999b) by way of rendering the enteric pathogens less resistant to garlic and other antimicrobials.

The highlight of this study was that raw garlic extract is a more effective antimicrobial agent than antibiotics currently in use. Garlic is followed closely by some antibiotics, such as CPX, whose similar mechanism of action is the inhibition of DNA and or RNA syntheses. Secondly, the effect of garlic extract is most pronounced on enteric bacterial pathogens. The absence of resistance to garlic enhances its ability to effectively act against even highly resistant bacterial strains, such as *Enterococcus* and *Pseudomonas aeruginosa*. It therefore appears attractive that antibiotics that affect DNA and RNA syntheses could form an effective combination with garlic.

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