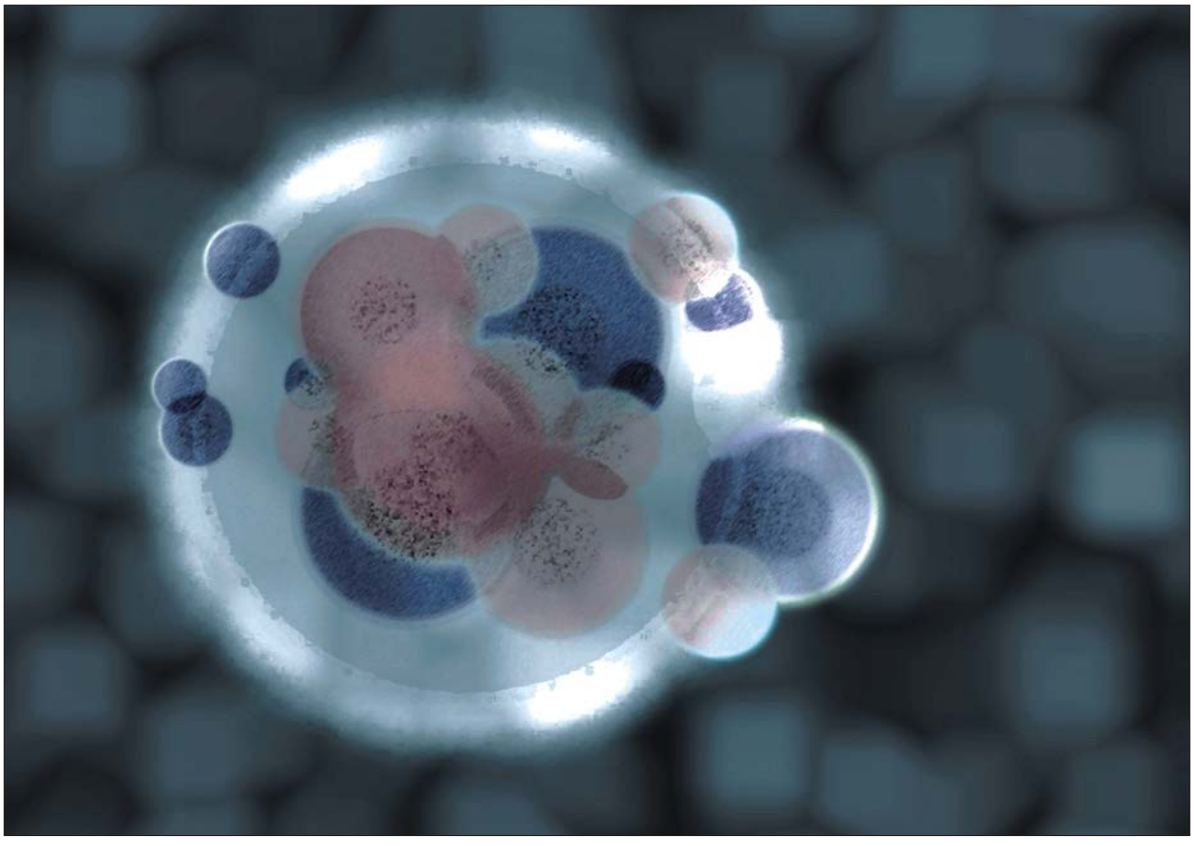

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In vitro Antibacterial Activity of Garlic Against Isolates of *Acinetobacter* sp.

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Abstract: The aim of present study is the evaluation of the antibacterial activity of chloroformic garlic extracts on isolates of *Acinetobacter*. A total of 20 isolates were collected from burn wounds at different hospitals in Tehran, Iran. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. Antibacterial activity of garlic extracts was measured by Minimum Bactericidal Concentrations (MBCs). Seventy five percent of isolated strains showed resistance to at least 12 antibiotics or more and all the isolates were Multi-Drug Resistant (MDR) isolates. The average MBCs of chloroformic garlic extract and Allicin content against all isolates of *Acinetobacter* sp. was 3.28±2.73 and 5.57±4.63 μg mL^{-1}, respectively. Present study suggests that garlic chloroformic extract has significant bactericidal action on multi-drug resistant strains of *Acinetobacter*.

Key words: Garlic, allicin, MBC, *Acinetobacter*, antibacterial, antibiotic

INTRODUCTION

The therapeutic effects of garlic include beneficial effects on the cardiovascular system, antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and hormone-like effects (Jonkers *et al*., 1999).

Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Historically, garlic has been used worldwide to fight bacterial infections. Allium vegetables, particularly garlic exhibit a broad antibiotic spectrum against both gram-positive and gram-negative bacteria (Sivam, 2001). It has been showed that pathogenic intestinal bacteria, which are responsible for diarrhea in humans and animals, are more easily inhibited by garlic than the normal intestinal flora (Caldwell and Danzer, 1988; Kumar and Sharma, 1982; Rees *et al*., 1993).

Garlic is active even against organisms that have become resistant to antibiotics (Ariga and Seki, 2006; Harris *et al*., 2001) and the combination of garlic extracts with antibiotics leads to partial or total synergism (Didry *et al*., 1992; Betoni *et al*., 2006). Also garlic oil preparation showed good anti-tuberculosis activity in guinea pigs (Jain, 1998). There are different studies showed complete lack of resistance of bacteria to garlic, Also toxin production by the bacteria is prevented by garlic (Sivam, 2001).

Acinetobacter sp. is small, aerobic, gram-negative rods that prefer moist environments (Gerner-Smidt, 1995). They are also nonfermentative, oxidase negative, non motile, catalase positive saprophytes that could be distinguished from other bacteria by their lack of pigmentation (Ingram and Shewan, 1960). *Acinetobacter* sp. is usually considered to be opportunistic pathogens. They cause a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection and meningitis, especially in immunocompromised patients (Bergogne-Bérézin and Towner, 1996) and patients admitted in intensive care and burn units (Humphreys and Towner, 1997), where they are frequent causes of ventilator-associated pneumonia and of bacteraemias (Bergogne-Berezin and Towner, 1996). They are often multi-resistant to antibiotics, meaning that therapy and infection control are complicated (Henwood *et al*., 2002). These microorganisms may acquire resistance to many antimicrobial agents. During the last decades hospital-acquired infections involving multi-resistant *Acinetobacter* isolates have been reported, often in association with contamination of the hospital equipment or cross-contamination by the colonized hands of patient attending personnel (Bergogne-Bérézin *et al*., 1987; Bergogne-Bérézin and Towner, 1996; Stone and Das, 1985; Struelens *et al*., 1993; Tankovic *et al*., 1994).

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Many strains of *Acinetobacter* show high rate of resistance to several antibiotics and other kinds of antimicrobial agents (Henwood *et al*., 2002), so introducing of the new antimicrobial agents against this bacterium is one of the most important goals in treatment of such infections. However there is no study on investigation the antibacterial effects of garlic on these bacteria.

In this study we evaluated the antibacterial activity of garlic on 20 hospital isolates of *Acinetobacter*.

MATERIALS AND METHODS

Bacterial strains and culture media: A total of 20 strains were collected from clinical specimens from burn wards of hospitals in Tehran, Iran during a six months period between April and September 2006. The isolates were further processed by the standard methods to identify as the *Acinetobacter* sp. Strains were maintained for long storage on Skimmed milk medium (BBL) by adding 10% glycerol in -70°C, cultures were maintained for daily use on Nutrient Agar (BBL) slants on 4°C. The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) medium (Pronadisa) were used for detection of antibiotic resistance of strains and measurement of the MBC of garlic extracts against each strain.

Determination of the strains sensitivity to antibiotics: The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method (Bauer *et al*., 1966). To represent the different classes of antimicrobial agents commonly used for the treatment of *Acinetobacter* sp. Infections, we used piperacillin, Gentamicin, Ofloxacin, Ceftriaxone, Ciprofloxacin, Cephalotin, Ticarcillin, Kanamycin, Imipenem, Amikacin, Co-Trimoxazole, Ceftizoxime, ceftazolin, Carbenicillin (Hi-media, Mombay, India).

Preparation of garlic extract: A weighted amount of fresh garlic was purchased from Urmia, Iran. Dry garlic bulbs were peeled, crushed and homogenized using a blender mixer with two parts of distilled water. The aqueous extract was passed through a gauze pad to remove the larger particles and filtrated. Then chloroform (Sigma) was added to aqueous extract. We slowly mixed this solution in a decanter until completely mixed. The organic fraction was collected and chloroform was separated from garlic extract at 40°C. The chloroform was removed overnight yielding a yellow, concentrated, jelly substance with a strong garlic odor.

The resulting paste was stored at -20°C until further use (Shapoury *et al*., 2006). Samples were dried in oven at 50°C to a constant weight and found to contain 125 mg (equivalent dry weight) of garlic extract per mL.

The concentration of allicin in each preparation was determined spectrophotometrically by reacting with the thiol, 4- mercaptopyridine (Sigma) (Miron *et al*., 2002).

Determination of antimicrobial activity of garlic chloroformic extract: The frozen bacterial strains were thawed and inoculated on nutrient agar medium and then cultured overnight at 36±0.5°C. The bacteria were suspended in 10 mL of sterile buffer saline and used as inoculate within 1 h after adjustment. One milliliter of garlic extract (adjusted to containing 100 mg) were added to 1 mL of MHB. The solution was diluted serially in 8 stages.

Bacterial inoculate were added to serial dilutions of garlic extract, with final bacterial concentrations of (1.5×10^6 cell mL^{-1}). Antibacterial activity was measured by determining MBC by culturing on MHA medium in a sterile Petri dish. Five microliter of each tube streaked on MHA plates, the highest dilution that inhibits bacterial growth on MHA after overnight incubation was taken as MBC (Tiwari *et al*., 2005; Sahm and Weissfeld, 2002).

RESULTS

A total of 20 *Acinetobacter* sp. isolates were collected from specimens submitted to the clinical microbiology laboratories of selected hospitals in Tehran, Iran. Strains were isolated from burn wounds of admitted patients.

Sensitivity of isolates of *Acinetobacter* to garlic extract: The average MBCs of the chloroformic garlic extract and Allicin against all strains of *Acinetobacter* sp. were 3.28±2.73 mg mL^{-1} and 5.57±4.63 μg mL^{-1}, respectively (Table 1).

Sensitivity of bacterial isolates to antibiotics: Seventy five percent of isolates showed resistance to at least 12 antibiotics or more, all the strains were multi-drug resistant strains (Table 2). Seven antibiotypes were recognized for all the isolated strains (Table 3). The average MBCs of garlic extract were calculated for each antibiotype separately, antibiotype 1 showed highest resistance to antibiotics (only sensitive to Imipenem) (Table 3), this antibiotype did not show highest resistance to garlic chloroformic extract. Antibiotype 7 is the most sensitive antibiotypes to antibiotics, but this antibiotype is not the most sensitive antibiotype to garlic chloroformic extract. Antibiotypes 4 and 2 are the most resistant antibiotypes to garlic chloroformic extract, respectively (Table 4).

Table 1: The average of minimum bactericidal concentrations for isolated strains of *Acinetobacter*

No. of strains	Dilution of garlic extract	The average MBCs of garlic extract for each strain (mg mL^{-1})	The average of allicin content (μg mL^{-1})
1	1/4	12.5	21.25
3	1/8	6.25	10.62
6	1/16	3.12	5.31
10	1/32	1.56	2.56
Total = 20	-	3.28±2.73	5.57±4.63

Table 2: Resistant percent to different antibiotics of 20 strains of *Acinetobacter* isolated from burn infections in a six months period (April-September 2006) from Tehran's hospitals

Antibiotic	Resistance (%)
Piperacillin (Pc)	90
Gentamicin (G)	70
Ofloxacin (Of)	95
Ciprofloxacin (Cf)	95
Cephalotin (Ch)	60
Ticarcillin (Ti)	95
Kanamycin (K)	95
Imipenem (I)	15
Amikacin (Ak)	50
Co-Trimoxazole (Co)	80
Ceftizoxime (Ce)	75
Ceftazolin (Cz)	100
Carbenicillin (Cb)	95

Table 3: Seven different Antibiotypes were recognized in 20 isolated strains. As Shown, 45% of strains belong to Antibiotype 1 (only sensitive to Imipenem)

Antibiotypes	Percent
PIP^R G^R OF^R CI^R CF^R CH^R TI^R K^R I^S AK^R CO^R CK^R CZ^R CB^R	45
PIP^R G^R OF^R CI^R CF^R CH^R TI^R K^R I^S AK^S CO^R CK^R CZ^R CB^R	15
PIP^R G^R OF^R CI^R CF^S CH^R TI^R K^R I^R AK^S CO^R CK^R CZ^R CB^R	10
PIP^R G^R OF^R CI^R CF^R CH^R TI^S K^R I^S AK^R CO^R CK^R CZ^R CB^R	5
PIP^R G^S OF^R CI^R CF^S CH^R TI^R K^R I^S AK^S CO^R CK^S CZ^R CB^R	5
PIP^R G^S OF^R CI^R CF^S CH^R TI^R K^R I^S AK^S CO^S CK^S CZ^R CB^R	15
PIP^S G^S OF^S CI^S CF^S CH^R TI^R K^S I^S AK^S CO^S CK^S CZ^R CB^R	5
Total	100

Table 4: The average MBCs of garlic extract and Allicin content for each antibiotype

Antibiotype	Average MBCs of garlic extract (mg mL^{-1})	Allicin content (μg mL^{-1})
1	1.90	3.24
2	6.25	10.62
3	2.34	3.98
4	12.50	21.25
5	3.12	5.31
6	2.60	4.42
7	1.56	2.65

DISCUSSION

The control of hospital acquired infection caused by multi-drug resistant gram negative bacilli has proven to be a particular problem in under developing as well as developed countries.

In the past few decades there was an increase in the importance of strictly aerobic gram negative bacilli, including *P. aeruginosa* and *Acinetobacter* sp.

Acinetobacter play a significant role in colonization and infection of patients admitted to hospitals. They have been implicated in a variety of nosocomial infections, including bacteremia, urinary tract infection, secondary meningitis and nosocomial pneumonia. Treatment of such infections are often extremely difficult because of high resistance of these bacteria to the major groups of antibiotics, on the other hand, *Acinetobacter* have a significant capacity for long-term survival in the hospital environment with enhanced opportunities for transmission between patients (Bergogne-Bérézin and Towner, 1996), so it seems reasonable to explore new sources of natural compounds with antibacterial activity against *Acinetobacter* sp.

Herbal drugs are continually tested and have been used to treat different kinds of disease as well as infectious disease worldwide (Moore and Atkins, 1977). Garlic has been reported to inhibit many species of bacteria including *Aerobacter*, *Aeromonas*, *Bacillus*, *Citrella*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Escherichia*, *Klebsiella Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella*, *Staphylococcus*, *Streptococcus* and *Vibrio* (Sivam, 2001). According to the study of Delaha and Garaqusi (1985) 1.67 mg mL^{-1} of chloroformic extracted garlic has an inhibitory effect on growth of *M. tuberculosis*, however in this study the average MBCs of garlic extract and Allicin content against all strains of *Acinetobacter* were 3.28±2.73 mg mL^{-1} and 5.57±4.63 μg mL^{-1}, respectively (Table 1).

In this study we evaluated the average MBCs of garlic extract for each antibiotype separately (Table 4), present results showed that there is not direct relations between the amount of sensitivity to garlic chloroformic extract and antibiotics of isolates, it means that multi-drug resistant strains of *Acinetobacter* with high resistance to antibiotics may be sensitive to low concentrations of garlic extracts.

Present study is the first study that examined the antibacterial effects of chloroformic garlic extract on the burn isolates of *Acinetobacter*, the present study shows that chloroformic garlic extract has significant activity with bactericidal action on multi-antibiotic resistant strains of *Acinetobacter*.

In conclusion garlic extract is a safe agent may be used as an anti-*Acinetobacter* agent against antibiotic resistant strains. However more studies on numerous strains of *Acinetobacter* isolated from different clinical specimens as well as in vivo studies should be done for confirmation of the antibacterial effects of garlic extracts for treatment of *Acinetobacter* caused infections.

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