African Journal of Microbiology Research Vol. 6(21), pp. 4475-4480, 9 June, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.540
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Inhibitory effect of aqueous garlic (*Allium sativum*) extract against clinical isolates of *Salmonella typhi*

Abdul Hannan[1], Kanwal Rauf[1], Muhammad Ikram Ullah[2], Tahir Naeem[3], Mehwish Raja[1], Muhammad Usman Qamar[1], Romeeza Tahir[4] and Mehwish Saba[1]

[1]Department of Microbiology, University of Health Sciences, Lahore-Pakistan.
[2]Department of Biochemistry, University of Health Sciences, Lahore-Pakistan.
[3]King Saud University Hospital Riyadh, Saudi Arabia.
[4]Department of Immunology, University of Health Sciences, Lahore-Pakistan.

Accepted 6 February, 2012

Typhoid Fever is a systemic disease which is more prevalent in under-developed countries. Drug resistance has been developed against antibiotics used for the treatment of typhoid. It is very important to set the basis of alternative medicines for management of typhoid. Garlic is one of the natural plants being used as spicy food and folk medicine. Various beneficial therapeutic effects of garlic have been documented including anti-microbial, hypolipodemic and anti-oxidant. The objective of present study was to explore anti-bacterial activity of aqueous garlic extract against *Salmonella typhi.* A total of 50 clinical isolates of *S. typhi* including 30 multi-drug resistant (MDR) and 20 antibiotic sensitive isolates were investigated to check the inhibitory effects of garlic extract; screening was done by agar well diffusion assay and minimal inhibitory concentration (MIC) was performed by agar dilution technique. MIC of garlic extract ranged from 18-22 mg/ml; showing the inhibitory activity of garlic extract against *S. typhi* isolates. It is worth describing that garlic might be utilized as anti-typhoid agent after determining its pharmacokinetics and pharmacodynamics.

Key words: Antibacterial activity, garlic extract, *Salmonella typhi,* minimal inhibitory concentration (MIC), agar dilution.

INTRODUCTION

Typhoid fever still causes substantial illness and deaths in many parts of the world, especially in developing nations (Ochiai et al., 2005). According to a report published WHO bulletin in 2000, the estimated global incidence of typhoid fever was about 21.6 million and mortality rate was up to 216,000 per year. The highest incidence rate of typhoid fever (>100/100,000 cases/year) has been reported in south-central Asia and south-east Asia (Crump et al., 2004). In 2008, it was reported that the incidence of typhoid fever is 451/100,000 in Pakistan (Kothari et al., 2008).

Multi drug resistant (MDR) *Salmonella typhi* showed resistance to all three first line drugs {Ampicillin (AMP), Chloramphenicol (C), and Trimethoprim-sulfamethoxazole (SXT)} (Ackers et al., 2000). MDR *S. typhi* emerged somewhere in the mid 1990s and are now reported in different regions of the world (Ward and Thelfall, 2001; Parry et al., 2002). Under these circumstances, third generation cephalosporin namely, ceftriaxone (CRO) appears to be the most reliable choice for MDR and nalidixic acid resistant isolates of *S. typhi* (NARST) (Bhutta, 2006). In 1999, high level resistance to CRO (MIC 64 mg/L) has been reported from Bangladesh (Parry et al., 2002; Saha et al., 1999). Recently extended spectrum beta lactamase (ESBL) production has been reported in *S. typhi* isolate isolated from the 54 year old Dutch man returned from Philippines (Naiemi et al., 2008).

This continuous spread of MDR pathogens and cost-effectiveness of drug regimen has become a serious threat to public health and infection control practitioners. The multiple and repeated difficulties with antibiotics has prompted research to explore alternate agents.

*Corresponding author. E-mail: kanwal.rauf@gmail.com. Tel: +92334-4153248.

Phytotherapeutic agents like garlic (*Allium sativum*) which is frequently used in alternative medicine has gained immense interest in medical literature (Iwalokun et al., 2004). Recently, garlic has been found to be an effective agent for its application as anti-tumor, anti-oxidant, anti-viral, anti-fungal, anti-microbial, anti-thrombotic, anti-inflammatory, hypoglycemic, immune modulatory effects (Amagase et al., 2001).

Garlic holds up the highest concentration of sulfur compounds like allicin and others having biological activities. These are responsible for not only its pungent smell but also for its medicinal value (Kemper, 2000). The thiosulfate allicin has effective anti-microbial properties and other non-sulphur constituents like proteins, saponins and phenolic compounds may also contribute to its anti-microbial activity (Corzo-Martinez et al., 2007).

Garlic is present in various forms namely, raw juice, garlic oil, garlic powder, and many types of extracts like, aged garlic extract, aqueous garlic extract (AGE), methanolic extract, ethanolic extract and many more (Jabar and Al-Mossawi, 2007). Different garlic extract preparations demonstrated their *in-vitro* activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium* and even acid-fast bacilli (AFB) such as *Mycobacterium tuberculosis* (MTB) (Uchida et al., 1975). It is also effective against antibiotic resistant isolates like methicillin-resistant *Staphylococcus aureus* (MRSA) as well as other MDR enterotoxicogenic isolates of *Escherichia coli* (ETEC), *Salmonella goldcoast* and *Klebsiellae* (Jabar and Al-Mossawi, 2007) and also MDR MTB (Hannan et al., 2011).

Garlic having multiple biological properties; inspired to investigate local cultivated garlic (small cloves) for its anti-typhoid effects against sensitive and MDR *S. typhi* isolates which might be helpful in combating this public health concern issue.

MATERIALS AND METHODS

Prior to the start of the study, approval was obtained from the Ethical Committee, University of Health Sciences, Lahore, Pakistan.

Bacterial isolates

Fifty clinical isolates of *S. typhi* were provided by the Department of Microbiology, Armed Force Institute of Pathology, Rawalpindi (AFIP), Army Medical College Rawalpindi, Pakistan (AMC) and Microbiology Department of Sheikh Zayed Medical Complex, Lahore (SZH).

Confirmation of isolates

These isolates were reconfirmed by various microbiological techniques namely, Gram stain, catalase test oxidase test and API 20E. Antibiotic profile has been performed by Kirby-Bauer disk diffusion method and the interpretation of the susceptibility was done according to interpretation break point, given in Clinical Laboratory Standards Institute (CLSI) 2009 guidelines (Wikler et al., 2009).

Aqueous garlic extract (AGE) preparation

Fresh garlic was purchased from local market. The aqueous garlic extract was prepared according to “Iwalokun BA” method. The cloves were separated and peeled to obtain the edible portion. Fifty Gram of the edible portion was chopped and homogenized in 100 mL autoclaved distilled water in a blender. The homogenate was filtered through 25 µm pore-size (Millipore) filter paper (Iwalokun et al., 2004).

Storage of extract stock solution

The stock concentration of 50 g/100 mL or 500 mg/mL of garlic extract was obtained and stored at 4°C in sterile screw cap vial (50 mL).

Anti-bacterial testing

The extract was screened against an isolate of MDR *S. typhi*, (AMC-3) by agar well diffusion assay. *Staphylococcus aureus,* ATCC (25923) and *S. typhimurium*, ATCC (14028) were used as quality control. The subculture isolates were adjusted to 0.5 McFarland standard and lawned on Mueller Hinton (MH) agar. Four serial dilutions (1/2; 250 mg/ml, 1/4; 125 mg/ml, 1/8; 62.5 mg/ml, 1/16; 31.25 mg/ml) were prepared from the stock solution of aqueous garlic extract in normal saline. Then stock solution of extract and its serial dilutions were filled in precut wells made in agar with 6 mm diameter sterile steel borer. The plates were incubated for overnight at 35-37°C. Bacteria showing a clear zone of more than 12 mm were considered to be inhibited. Two controls were run simultaneously, distilled water (diluent) was used as negative control and Ciprofloxacin (5 µg) serve as positive control. This procedure was performed in duplicate.

Determination of Minimal Inhibitory concentration (MIC)

MIC was determined by agar dilution method. AGE was mixed with sterilize MH agar at 50°C to achieve the desired concentrations from 0.5 to 30 mg/ml. The grid was filled with 500 µl of 0.5 McFarland standard bacterial suspensions. Then it was placed side by side with extract incorporated plates in a multi-inoculator. All plates were punched with 3 µl of bacterial suspensions and simultaneously 35 isolates were inoculated at single plate including the ATCC strains. Then the plates were incubated for over night at appropriate temperature. Two control plates were also set up in parallel. The positive control contains MH agar plate without extract and inoculated with all isolates to confirm the viability of the cultures while the sterility control contains un-inoculated MH. The lowest concentration of AGE at which all bacterial isolates were inhibited was MIC.

Data analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 17.0.

RESULTS

According to the anti-microbial sensitivity profile results,

Table 1. Anti-microbial susceptibility pattern of *S. typhi* to first-line anti-typhoid drugs.

S. typhi isolates	No (%)
MDR isolates*	30 (60)
Sensitive isolates	20 (40)
Total	50 (100)

*MDR; Multi-drug resistant.

out of 50 *S. typhi* isolates 30 isolates (60%) were MDR *S. typhi* while 20 (40%) were sensitive to all three first line drugs namely C, SXT and AMP (Table 1).

Anti-bacterial activity of AGE

The sizes of inhibition zones were inversely proportional to the increase in dilution of AGE. The most sensitive strain was ATCC *S. typhimurium* (14028) which showed the largest zone (16±1 mm) of inhibition at the highest dilution (1/16; 31.25 mg/ml), followed by MDR *S. typhi* (12.5±1.5 mm). The AGE did not exhibit any zone against ATCC *S. aureus* (25923) at the same dilution. MDR *S. typhi* isolate showed zone of inhibition >12 mm at all serial dilutions except 1/16 (31.25 mg/ml). The results are given in Table 2 and Figure 1.

MIC of AGE

Maximum numbers of sensitive isolates (n=9) were inhibited at 18 mg/mL while MDR *S. typhi* isolates (n=9) at 22 mg/mL (Table 3). Table 4 represents MIC range of garlic extract (18-22 mg/ml) on 50 isolates of *S. typhi*. MIC_{100} for sensitive and MDR isolates was same as 22 mg/ml while MIC_{90} for sensitive and MDR isolates was > 20 and > 21 mg/ml respectively. Figure 2 showed the cumulative percentage of MDR *S. typhi* which is 13, 30, 50, 70 and 100% at concentrations of 18, 19, 20, 21 and 22 mg/ml of garlic extract respectively.

DISCUSSION

The widespread and injudicious use of antimicrobial agents in human has led to the development of extensive resistance amongst bacterial pathogens. Bacterial resistance is the major cause of failure of antibiotic therapy requiring additional courses of protracted antibiotic usage and in effect setting up a vicious cycle (Hopkins et al., 2005).

In the present study both Gram negative rods {*S. typhimurium* (ATCC 14028), MDR *S. typhi*} were more sensitive to AGE than *S. aureus* (ATCC 25923). This demonstrates that Gram positive organisms may be better protected naturally against the action of garlic extract because of the presence of thick layer of peptidoglycan which impairs the access the antibacterial agent (allicin) (Bakri and Douglas, 2005; Indu et al., 2006).

A comparable study done on AGE, also demonstrated the zone of inhibition of 23.8±1.5 mm against 10 MDR *S. typhi* isolates (Iwalokun et al., 2004). In contrast, a Nigerian study demonstrated different results which showed that no zone of inhibition was formed by aqueous extract against *S. typhi* (Ekwenye and Elegalam, 2005). This variation may be due to the fact that garlic composition depends upon many factors like origin, age, storage conditions and method used for the preparation of extract which may in turn its activity (Sivam, 2001).

In case of MIC, more MDR isolates were inhibited at 22 mg/ml than sensitive isolates, illustrating that some type of resistant may also exists in MDR isolates. But in contrast to this observation both MDR (n=30) and sensitive (n=20) isolates were inhibited within same range that is 18-22 mg/ml. It might be due to the difference in mechanism of action of garlic; antibiotics have a single mode of action and it is 1000-fold easier to develop resistance against antibiotics drugs. On the other hand garlic has multiple mechanisms due to its various constituents that give their effects simultaneously (Jabar and Al-Mossawi, 2007; Bakri and Douglas, 2005). This showed that garlic was equally effective against MDR and sensitive isolates.

In the present study, the MIC range for 50 *S. typhi* isolates came out to be 18 - 22 mg/ml, by agar dilution method. In Nigerian study, MIC range for 10 MDR *S. typhi* isolates was 16-27 mg/ml (Iwalokun et al., 2004). However, another study proved that *S. typhi* isolate was not inhibited at even 1000 mg/ml (Ekwenye and Elegalam, 2005). The most probable explanation for these differences are the amount and composition of organo-sulfur compounds which vary with different species of garlic (Arora and Kaur, 1999).

The basic ground for selecting AGE for this study was because of high concentration of allicin (antibacterial component) as compared to other extracts (Jabar and Al-Mossawi, 2007). AGE could be conveniently utilized *in-vivo* studies (Kemper, 2000). The major drawbacks in its use are that the amount of allicin decreases with time. Thus it cannot be preserved for longer time and its antibacterial properties also decline with the age of extract. Therefore, fresh AGE should be prepared each time before its use (Pranoto et al., 2005).

One of the disadvantages in evaluating antibacterial activity of AGE is lack of standardization in techniques being used by the scientists. This gives rise to marked difference in results obtained.

The most likely rationalization for this difference is may be due to existence of variation in the component of different origin. A study by Lawson et al., (1991) established that the garlic cropped in China may have

Table 2. Anti-bacterial activity of AGE in agar well diffusion assay (Zone of inhibition).

Dilutions of AGE (mg/mL)	Zones of inhibition (mm)		
	MDR *S. typhi* isolates	*S. aureus* ATCC (25923)	*S. typhimurium* ATCC (14028)
Stock solution (500)	34±1	38±1.5	36±2
1/2(250)	26±1	32±1	31±1.5
1/4(125)	21.5±1.5	25±1	29±1.5
1/8(62.5)	16.5±1	-	25±1
1/16(31.25)	12.5±1.5	-	16±1

*AGE; Aqueous garlic extract, MDR; multi-drug resistant; ATCC; American type culture collection.

Table 3. Inhibition of MDR and non-MDR *S. typhi* isolates with AGE (mg/mL).

AGE (mg/mL)	Sensitive isolates (n=20)	MDR isolates (n=30)
18	09	04
19	04	05
20	04	06
21	02	06
22	01	09

Table 4. MIC of AGE against MDR (n=30) and sensitive (n=20) *S. typhi* isolates.

S. typhi isolates	MIC range (mg/ml)	MIC_{50} (mg/ml)	MIC_{90} (mg/ml)	MIC_{100} (mg/ml)
MDR isolates	18-22	20	>21	22
Sensitive isolates	18-22	>18	>20	22

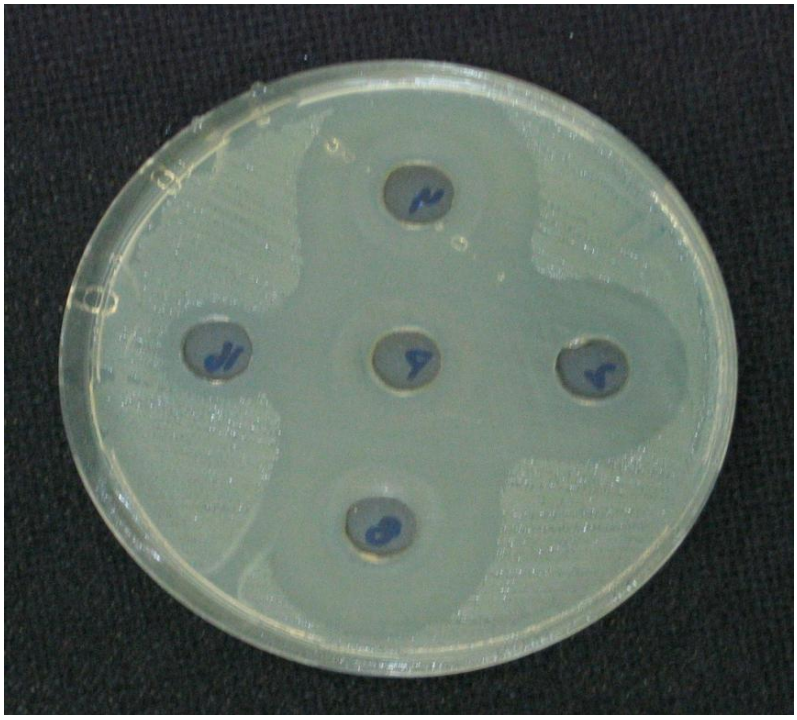

Figure 1. Anti-bacterial activity of garlic showing zone of inhibition on plate.

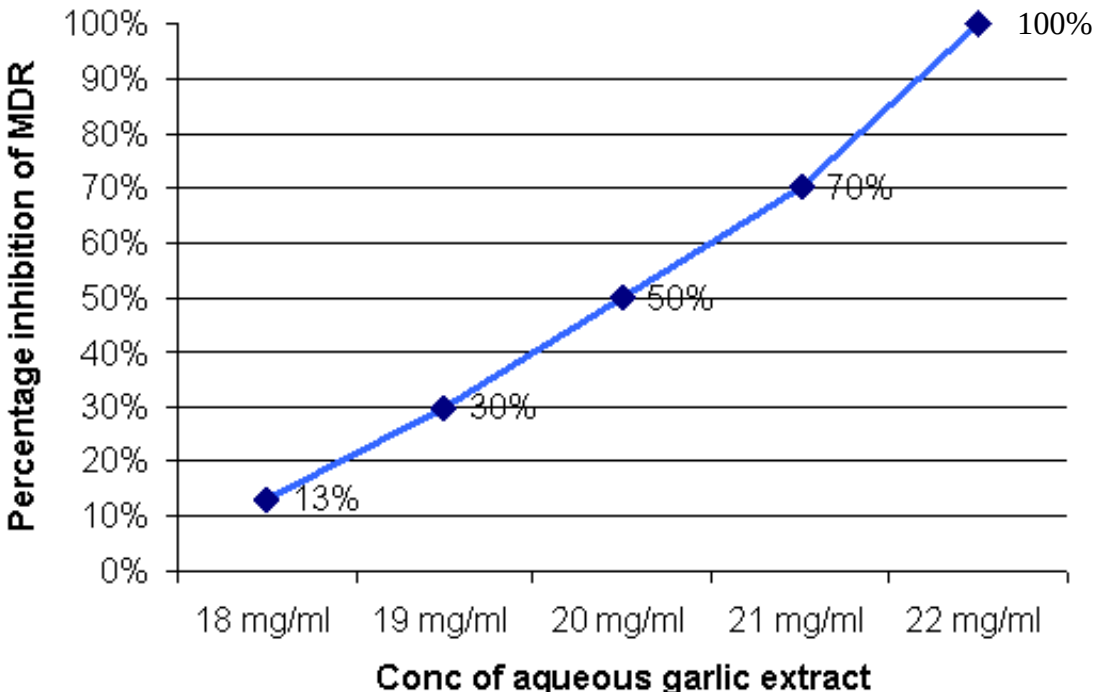

Figure 2. Cumulative percentage of multi-drug resistant *S. typhi* (n=30) inhibited at different concentrations of garlic extract.

twice allicin as much as in Europe or United States. Another reason which also effected upon results is type of methodology adopted for the assessment of screening and MIC of the extract. A number of factors namely, depth of agar, pH, lag-time required for the diffusion of extract, size of inoculum and length of incubation may influence the MIC of the extract (Pranoto et al., 2005).

The shortcomings in different techniques demand standardization so that inter-study outcome can be safely measured up. Lastly it is important to develop guidelines for all procedure adopted in evaluating antibacterial activity of AGE. It is also a need of hour to investigate extracts of allium species of different geographical locations for the most active ingredients responsible for their antibacterial activity.

Conclusion

Aqueous garlic extract was found to be inhibitory against the isolates of *S. typhi*. It is worth describing that garlic might be utilized as anti-typhoid agent after determining its pharmacokinetics and pharmacodynamics.

ACKNOWLEDGEMENT

We are grateful to University of Health Sciences, Lahore Pakistan for financial and logistic support for this research project.

REFERENCES

Ackers M, Puhr ND, Tauxe RV, Mintz ED (2000). Laboratory-base surveillance of *Salmonella* Serotype Typhi infections in the United States. JAMA., 283: 2668-2673.

Amagase H, Petesh BL, Matsuua H, Kasuga S, Itakura Y (2001). Intake of garlic and its bioactive components. J. Nutr., 131: 955S-962S.

Arora DS, Kaur J (1999). Antimicrobial activity of spices. Int. J. Antimicrob. Agents, 12: 257-262.

Bakri IM, Douglas CWI (2005). Inhibitory effect of garlic on oral bacteria. Arch. Oral. Biol., 50: 645-651.

Bhutta ZA (2006). Current concepts in the diagnosis and treatment of typhoid fever. BMJ., 333: 78-82.

Corzo-Martinez M, Corzo N, Villamiel M (2007). Biological properties of onion and garlic. Trends. Food. Sci. Technol., 18: 609-625

Crump JA, Luby SP, Mintz ED (2004). The global burden of typhoid fever. Bull. World. Health. Organ., 82: 346-353.

Ekwenye, Elegalam (2005). Antimicrobial activity of ginger (*Zingiber officinale Roscoe)* and garlic (*Allium sativum*) extracts on *Escherichia coli* and *Salmonella Typhi*. Intl. J. Mol. Med. Adv Sci., 1: 411-416.

Hannan A, Ikram Ullah M, Usman, Hussain S, Absar M, Javed K (2011). Anti-Mycobacterial activity of Garlic (*Allium Sativum*) against Multi-Drug Resistant and non-Multi-Drug Resistant *Mycobacterium Tuberculosis.* Pak. J. Pharm. Sci., 24(1): 81-85.

Hopkins KL, Davies RH, Threlfall EJ (2005). Mechanism of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. Int. J. Antimicrob. Agent, 25:358-373.

Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G (2006). Antimicrobial activity of some of the south-indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophilla*. Braz. J. Microbiol., 37: 153-158.

Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J (2004). *In-vitro* anti-microbial properties of aqueous Garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. J. Med. Food, 7: 327-333.

Jabar MA, Al-Mossawi A (2007). Susceptibility of some multiple resistant bacterial to garlic extract. Afr. J. Biotechnol., 6: 771-776.

Kemper KJ (2000). Garlic (Allium sativum). Revised March. Available from URL: www.mcp.edu/herbal/default.htm. Accessed January 24, 2011.
Kothari A, Pruthi A, Chugh TD (2008). The Burden of Enteric Fever. J. Infect. Dev. Count., 2: 253-259.
Lawson LD, Wang ZJ, Hughes BG (1991). Identification and HPLC quantitation of the sulfides and di-alk(en)yl thiosulfinates in commercial garlic products. Plant. Med., 57: 363-370.
Naiemi NA, Zwart B, Rijnsburger MC, Roosendaal R, Debets-Ossenkopp YJ, Mulder JA (2008). Extended-Spectrum-Beta-Lactamase production in a Salmonella enterica serotype Typhi strain from the Philippines. J. Clin. Microbiol., 46: 2794-2795.
Ochiai RL, Wang XY, Seidlein L, Yang J, Bhutta ZA, Bhattacharya SK (2005). *Salmonella* Paratyphi A rates, Asia. Emerg. Infec. Dis., 11:1764-1766.
Parry C, Hien TT, Dougan G, White NJ, Farrar JJ (2002). Typhoid Fever. N. Engl. J. Med., 347:1770-1782.
Pranoto Y, Rakshit SK, Salokhe VM (2005). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassiumsorbate and nisin. LWT., 38: 859-865.
Saha SK, Talukder SY, Islam M, and Saha S (1999). A highly ceftriaxone-resistant *Salmonella typhi* in Bangladesh. Pediatr. Infect. Dis. J., 18:387.
Sivam GP (2001). Protection against Helicobacter pylori and other bacterial Infection by garlic. J. Nutr., 131:1106S-118S.
Uchida Y, Takahashi T, Sato N (1975). The characteristics of the antibacterial activity of garlic. Jpn. J. Antibiotics, 28:638-642.
Ward LR, Thelfall EJ (2001). Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi United Kingdom. Emerg. Infec. Dis., 7: 448-450.
Wikler MA, Cockerill FR, Craig WA, Dudley MN, Eliopoulos GM, Hecht DW (2009). Clinical and Laboratory Standards Institute (CLSI), 19th Informational Supplement., 29(3): M02-A10.