

Full Length Research Paper

Combined activity of garlic and nitrofurantoin against *Escherichia coli* and *Enterococcus* species recovered from urinary tract infections

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Nitrofurantoin represents an attractive choice for empirical treatment of acute uncomplicated cystitis, and for long term prophylaxis against recurrent urinary tract infections. However, rather rare but severe adverse effects related to cumulative drug doses may occur. Bioactive compounds of plant origin combined with antibiotics can increase the sensitivity of microbial cells to such antibiotics. Garlic has antimicrobial effects against a wide range of microorganisms. The minimum inhibitory concentrations (MICs) of each of nitrofurantoin and garlic alone, and in combination were determined against 17 extended spectrum β -lactamase (ESBL) producing *Escherichia coli* and 24 *Enterococcus* spp. urinary isolates. When grown as planktonic cells, none of the *E. coli* isolates demonstrated resistance towards nitrofurantoin, whereas only one (4.2%) *Enterococcus* isolate was resistant. Garlic showed an inhibitory effect on planktonically grown ESBL producing *E. coli* and *Enterococcus* spp. with varying MICs. Each of nitrofurantoin and garlic tested alone showed an increase in the MICs for biofilm grown isolates compared to their planktonic counterparts. However, the combination of both agents led to significant decline of the MICs, whether for planktonic or biofilm forms, resulting in either synergy or addition. In conclusion, garlic enhanced the antibacterial activity of nitrofurantoin towards the tested urinary isolates.

Key words: Antimicrobial combination, biofilm, checkerboard assay, *Enterococcus* spp., extended spectrum β -lactamase (ESBL)-producing *Escherichia coli*, garlic extract, nitrofurantoin.

INTRODUCTION

Urinary tract infections (UTIs) are the commonest encountered in clinical practice (Butt et al., 2004), and many of the causative micro-organisms tend to respond to the urinary tract environment by biofilm formation producing chronic and often intractable infections (Subramanian et al., 2012). *Escherichia coli* is the most predominant pathogen causing community and nosocomially-acquired UTIs (Ejrnæs, 2011); which has become difficult to treat due to the increased prevalence of extended-spectrum β -lactamase (ESBL) production (Chaudhary and Aggarwal,

2004). On the other hand, UTIs due to Gram-positive bacteria are fairly uncommon; however they are usually caused by *Staphylococcus* spp. and enterococci (Bouza et al., 2001; Baral et al., 2013). Multi-drug resistance is very common among urinary enterococci, resulting in lack of oral treatment alternatives (Baral et al., 2013).

Nitrofurantoin is a synthetic antibacterial agent effective against most common Gram-negative and Gram-positive urinary tract pathogenic bacteria (Rafii and Hansen, 1998). The drug's susceptibility correlates with the reduction of

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nitrofurantoin inside the bacterial cell to highly reactive intermediates that attack the cell's ribosomal proteins, DNA and other macromolecules (Cetti et al., 2009). Fortunately, resistance to this drug has remained virtually low and unchanged since its introduction in 1953, making it an attractive choice for empirical treatment of acute uncomplicated cystitis (Gupta et al., 2011) and long term prophylaxis against recurrent UTIs (Karpman and Kurzrock, 2004; Cetti et al., 2009). However, severe but rather rare adverse reactions such as hepatotoxicity and neuropathy based on cumulative doses (Karpman and Kurzrock, 2004; Sharafadinzadeh et al., 2008) necessitate caution when issuing nitrofurantoin prescriptions and a need for changing the management protocols (Cetti et al., 2009).

Garlic (*Allium sativum*), an essential food ingredient worldwide, has long been known to have antibacterial, antifungal and antiviral effects. The main antimicrobial constituent of garlic, allicin, is generated by the enzyme alliinase when garlic is crushed (Lee et al., 2011). Allicin interacts with important thiol-containing enzymes as cysteine proteinases, alcohol dehydrogenase, as well as the thio-redoxin reductases, which are critical for maintaining the correct redox state within microorganisms (Ankri and Mirelman, 1999). Enhancement of the antibacterial activity of different antibiotic classes towards Gram-positive species, Gram-negative species and even *Mycobacterium tuberculosis* has been demonstrated in various combination studies with either crude garlic extract or allicin (Ankri and Mirelman, 1999; Jonkers et al., 1999; Cai et al., 2007). Relatively few side effects have been reported in humans using garlic and its preparations, mostly related to gastrointestinal discomfort and nausea (Banerjee and Maulik, 2002). On the other hand, high garlic concentrations have shown to be clastogenic in mice (Das et al., 1996), and have induced altered structure and function of the heart, liver and kidneys in rats when administered for prolonged periods (Banerjee and Maulik, 2002).

The aim of the present study was to examine the *in vitro* activity of garlic and its potential for synergy when combined with nitrofurantoin against urinary isolates of *E. coli* and enterococci.

MATERIAL AND METHODS

Bacterial isolates

The present study was conducted on non-duplicate urinary isolates belonging to *E. coli* and *Enterococcus* spp. Seventeen ESBL producing *E. coli* isolates recovered from UTIs and identified conventionally (Nataro et al., 2011) were included in the study. ESBL-expression was confirmed by the double disk synergy test according to the clinical laboratory standards institute (CLSI) document M100-S23 (CLSI, 2013). In addition, 24 enterococcal isolates which were recovered from patients with UTI and identified conventionally at first according to Teixeira et al. (2011) followed by the API-20 Strep system (Bio-Merieux, Marcy-l'Etoile, France) were included in the present study. All isolates were tested by the disc diffusion method (Patel et al., 2011) for their susceptibility towards antibiotics

used for urinary tract infections, namely; lomefloxacin (10 µg), norfloxacin (10 µg) and trimethoprim-sulphamethoxazole (1.25/23.75 µg); were tested against the *E. coli* isolates, whereas ciprofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg) and tetracycline (30 µg) were used for isolates of the *Enterococcus* spp. (CLSI, 2013). *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were also included in the study as quality control strains.

Biofilm production

The tissue culture plate method was adapted for quantitative detection of biofilm production according to Hassan et al. (2011) for *E. coli* and Marra et al. (2007) as well as Kouidhi et al. (2011) for enterococci. Overnight cultures from fresh agar plates were inoculated onto trypticase soy broth (Oxoid Ltd., Hampshire, England) with 1% glucose (w/v) and brain heart infusion broth (Oxoid Ltd., Hampshire, England) with 2% glucose (w/v) for *E. coli* and enterococci; respectively. After overnight incubation at 37°C, the broths were diluted 1:100 with their corresponding media. Then, 200 µl of these cell suspensions were inoculated into sterile 96 well flat bottom polystyrene tissue culture treated plates (Nunc, Roskilde, Denmark) and incubated 24 h at 37°C. Each isolate was tested in duplicates and uninoculated wells containing sterile broth served as negative control. Biofilms formed by *E. coli* were fixed by 2% sodium acetate and stained by 0.1% crystal violet, followed by removing excess stain by deionized water and drying the plates. On the other hand, enterococcal biofilms were stained by 0.2% crystal violet and washed 3 times with phosphate buffered saline (PBS), followed by extraction of the crystal violet bound to the biofilm with 200 µl of an 80:20 mixture of ethanol and acetone.

Biofilm formation was assessed spectrophotometrically using a microplate reader (Stat Fax-2100, Awareness) at 570 and 595 nm for *E. coli* and enterococci; respectively, whereby isolates were classified as non biofilm producers, weak producers or strong producers according to Christensen et al. (1985).

Garlic extraction

1 kg of garlic cloves was pulverized and soaked daily on 3 consecutive days in 500 ml ethanol at room temperature. The ethanol extract was concentrated under reduced pressure at 40°C to give a residue of 60 g. (Wu et al., 2012). The powder extract was weighed and dissolved in a measured amount of sterile distilled water to reach the desired concentration. Then, it was sterilized using Nalgene™ 0.45 µm syringe filter (Thermo Fisher Scientific, Langensfeld, Germany) and the concentration of the filtered extract was considered as 50% that of the pre-filtered one (Hindi, 2013).

Determination of the antimicrobial activity

The minimum inhibitory concentration (MIC) of nitrofurantoin (Macrofuran 50 mg capsules, KahiraPharm, Egypt) for isolates of *E. coli* and *Enterococcus* spp. was determined by the broth microdilution method (CLSI, 2013). The procedure was also adapted for testing serial dilutions of freshly dissolved garlic extract. The tested final nitrofurantoin concentrations ranged from 0.5 – 256 µg/ml dissolved in sterile dimethyl sulfoxide and the MIC breakpoint interpretation was done according to the CLSI guidelines (CLSI, 2013), whereas the tested final garlic concentrations ranged initially from 97.66 to 25,000 µg/ml., followed by extra sets of concentrations ranging from 37,500 to 150,000 µg/ml and from 50,000 to 200,000 µg/ml, when growth was observed throughout all the initial concentrations.

Moreover, the susceptibility of the biofilm grown *E. coli* and enterococci isolates towards each of nitrofurantoin and garlic was done by the microplate alamar blue assay (Flemming et al., 2009). Briefly, washed biofilms were subjected to treatment with the previously mentioned agents with the same prepared concentrations. Positive controls formed of untreated biofilms with only 200 μ l of Mueller-Hinton II broth (MHB) (Oxoid Ltd., Hampshire, England), and negative controls formed of only 200 μ l MHB with no added bacteria were included in the assay. After incubation at 37°C for 24 h, wells were washed twice with PBS, then the metabolic activity of the biofilms was quantified by adding 250 μ l MHB with 5% alamar blue (AbD Serotec, Oxon, UK) per well. The plates were shaken gently for 30 s, incubated for 1 h at 37°C and the absorbance was obtained at 570 and 600 nm. Then, the alamar blue percent reduction was calculated and the minimal biofilm inhibitory concentration was defined as the lowest drug concentration resulting in \leq 50% reduction and a blue well.

Assessment of the interaction between nitrofurantoin and garlic

The *in vitro* activity of nitrofurantoin and garlic in combination against each of *E. coli* and enterococci urinary isolates was tested by the checkerboard method (Isenberg, 2007).

Planktonic checkerboard assay

The range of concentrations was prepared according to the previously determined MIC of each agent for each isolate. Tested concentrations ranged from 0.03x MIC to 4.0x MIC for each agent. In addition, the MIC of each of nitrofurantoin and garlic alone was determined on different sides of the same plate. Positive controls (only bacterial inoculum and MHB) and negative controls (only MHB) were also included in each plate. The final concentration of the inoculum was 10⁶ CFU/ml. After incubation at 37°C for 24 h, the combined effect was analyzed by calculation of the fractional inhibitory concentration index (FICI) using the following equation: $FICI = (MIC \text{ of drug A in the combination} / MIC \text{ of drug A alone}) + (MIC \text{ of drug B in the combination} / MIC \text{ of drug B alone})$. The lowest FICI value obtained in the checkerboard test was considered to be representative of the interaction of the two agents against the respective isolate. FICI results for each combination were interpreted as synergy for $FICI \leq 0.5$, additive for $0.5 < FICI \leq 1$, indifferent for $1 < FICI < 4$ and antagonistic for $FICI \geq 4$ (Pillai et al., 2005).

Biofilm checkerboard assay

Formed biofilms were tested by the previously described microplate alamar blue assay, except that combinations were used instead of individual agents in the same manner as the planktonic checkerboard assay. Addition of alamar blue, calculation of its percent reduction and the FIC indices as well as their interpretation was done exactly as previously described.

Statistical analysis

Data were statistically described in terms of mean \pm standard deviation (\pm SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann Whitney U test for independent samples. Within each group, comparison of numerical variables was done using Wilcoxon signed rank test for paired (matched) samples. For comparing categorical data, Chi

square (χ^2) test was performed. Exact test was used when the expected frequency was less than 5. *P* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., released 2006. SPSS Statistics for Windows, version 15.0. Chicago, IL, USA).

RESULTS

The present study was conducted on 17 ESBL-producing *E. coli* urinary isolates, as well as 24 enterococcal urinary isolates; 21/24 (87.5%) *E. faecalis* and 3/24 (12.5%) *E. faecium*. Biofilm production was detected in 3/17 (17.6%) *E. coli* and 14/24 (58.3%) *Enterococcus* spp.; two out of which belonged to *E. faecium* and were weak biofilm forming (Table 1). *E. coli* ATCC 25922 was non-biofilm forming, whereas *E. faecalis* ATCC 29212 was strong biofilm forming.

Testing by the disc diffusion method showed that all the *E. coli* isolates (100%) were resistant to lomefloxacin and norfloxacin, whereas 14 out of the 17 isolates (82.4%) were resistant to trimethoprim-sulphamethoxazole. Concerning isolates belonging to the *Enterococcus* spp., resistance was least detected towards each of levofloxacin and norfloxacin (6/24; 25% each), followed by ciprofloxacin (7/24; 29.2%), then tetracycline (21/24; 87.5%). On the other hand, resistance towards nitrofurantoin was not detected among the *E. coli* isolates, but was detected in only one (4.2%) *Enterococcus* isolate. The antimicrobial activity of nitrofurantoin and garlic towards the tested urinary isolates when grown planktonically *in vitro* is summarized in Table 2. The MIC of nitrofurantoin for *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212 was 8 μ g/ml and 4 μ g/ml; respectively, whereas the garlic MIC was 12,500 and 25,000 μ g/ml; respectively.

There was a significant decline in the MIC values for each of nitrofurantoin and garlic when combined together against planktonic growth of all tested urinary isolates compared to their values when tested alone (Table 3). No antagonistic activity was demonstrated towards any of the tested isolates. The combination was synergistic for more *E. coli* isolates than enterococci (47% versus 41.7%; respectively), but with no statistical significance (*P* = 0.425). The FIC index for 50% and 90% of the *E. coli* and enterococci isolates was the same (Table 4). Combination of nitrofurantoin and garlic on *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212 resulted in an FICI of 0.75 (addition) and 0.375 (synergy); respectively.

Each of nitrofurantoin and garlic tested alone against the three biofilm forming *E. coli* isolates showed an increase in the MIC values compared to their corresponding planktonic values. Nevertheless, the combination of both agents on these sessile forms led to a decline of each agents' MICs, with the result of either synergy or addition (Table 5). On the other hand, the MIC of each of nitrofurantoin and garlic when tested alone against the biofilm forming enterococci (14 isolates) exceeded 512 and 800,000 μ g/ml; respectively. Accordingly, the combi-

Table 1. Biofilm production among the studied urinary isolates.

Specie	Biofilm production		
	-	+	+++
<i>E. coli</i> (n=17)	14 (82.4%)	1 (6%)	2 (11.8%)
<i>Enterococcus spp.</i> (n=24)	10 (41.7%)	7 (29.2%)	7 (29.2%)

(-) = no production; (+) = weak production; (+++) = strong production.

Table 2. Antimicrobial activity of nitrofurantoin and garlic against planktonic growth of *E. coli* and *Enterococcus spp.*

Specie	Nitrofurantoin Susceptibility			Nitrofurantoin MIC ($\mu\text{g/ml}$)			Garlic MIC ($\mu\text{g/ml}$)		
	S	I	R	Range	*MIC50	**MIC90	Range	*MIC50	**MIC90
<i>E. coli</i> (n=17)	11 (64.7%)	6 (35.3%)	0	8 - 64	32	64	6250-100,000	25,000	100,000
<i>Enterococcus spp.</i> (n=24)	20 (83.3%)	3 (12.5%)	1 (4.2%)	4 - 512	8	64	25,000-75,000	25,000	37,500

S = sensitive; I = intermediate; R = resistant. *MIC of 50% of the isolates; ** MIC of 90% of the isolates.

Table 3. Mean nitrofurantoin and garlic MIC values for the tested isolates when grown planktonically.

Specie	Nitrofurantoin Mean (\pm SD) MIC values ($\mu\text{g/ml}$)			P	Garlic Mean (\pm SD) MIC values ($\mu\text{g/ml}$)			P
	Alone	In combination			Alone	In combination		
	<i>E. coli</i>	39 \pm 20.373	13.53 \pm 8.762		*<0.001	37.8 \pm 31.1**	6.5 \pm 43.2**	
<i>Enterococcus spp.</i>	37.33 \pm 103.057	10.04 \pm 25.574	*<0.001	34.3 \pm 13.1**	10.8 \pm 10.9**	*<0.001		

*P value < 0.05 is significant; ** x1000

Table 4. Effect of nitrofurantoin and garlic combination on the tested urinary isolates in planktonic growth.

Species	FICI			Effect of combination			
	Range	*FICI 50	**FICI 90	Syn	Add	Ind	Ant
<i>E. coli</i> (n=17)	0.25-1.25	0.625	0.75	8 (47%)	8 (47%)	1 (5.9%)	0
<i>Enterococcus spp.</i> (n=24)	0.375-1	0.625	0.75	10 (41.7%)	14 (58.3%)	0	0

*FICI achieved by 50% of the isolates; **FICI achieved by 90% of the isolates. Syn = Synergistic; Add = additive; Ind = indifferent; Ant = antagonistic.

nation effect of nitrofurantoin and garlic could not be assessed on the sessile growth of such isolates.

DISCUSSION

UTIs represent a major public health problem (Butt et al., 2004). Biofilm-linked infections are particularly problematic, because biofilm associated bacteria can withstand host immune defenses, antibiotics, biocides and hydrogen shear forces far better than the corresponding planktonic bacteria (Hancock et al., 2010).

In the present study, biofilm production was detected in only 3/17 (17.6%) urinary ESBL producing *E. coli* isolates. Our results are almost comparable to the results of Novais et al. (2013), where 25.7% ESBL producing urinary *E. coli* isolates produced biofilm. Higher (52%) and lower (6%) results have been reported by Ghanwate (2012) and Ponnusamy et al. (2012); respectively. *E. coli* biofilms are often associated with long term persistence of the organism in the environment (Ponnusamy et al., 2012). This has been supported by observing that *E. coli* biofilm is not a relevant virulence factor for acute cystitis but is frequently described for catheter-associated, chronic and

Table 5. Effect of nitrofurantoin and garlic alone and combined on the three biofilm forming ESBL producing *E. coli* isolates

Isolate Number	Nitrofurantoin MIC ($\mu\text{g/ml}$)			Garlic MIC ($\mu\text{g/ml}$)			Effect of combination	
	Alone on planktonic growth	Alone on biofilm	In combination on biofilm	Alone on planktonic growth	Alone on biofilm	In combination on biofilm	FICI	Result
<i>E. coli</i> 16	64	128	64	100,000	200,000	50,000	0.75	Add
<i>E. coli</i> 28	32	64	8	25,000	200,000	50,000	0.375	Syn
<i>E. coli</i> 37	64	2048	16	6250	200,000	50,000	0.2578	Syn

Add = Additive; Syn = Synergistic.

recurrent UTIs (Norinder et al., 2012). An issue that was not addressed in this study was whether the patients, from whom the urinary specimens were collected, were catheterized or not, or whether they were suffering from acute, chronic or recurrent UTIs.

On the other hand, 14 out of 24 (58.3%) urinary enterococcal isolates produced biofilm in the current study. Our results are in agreement with the results of others (Sandoe et al., 2003; Seno et al., 2005). A higher rate for urinary enterococcal biofilm production reaching 74% has also been reported (Comerlato et al., 2013). Biofilm production by enterococci is important in its pathogenesis (Upadhyaya et al., 2011) and is multifactorial depending on a number of genes working together along with external factors (Comerlato et al., 2013).

ESBL producing *E. coli* causing UTIs have been primarily considered as multi-resistant organisms originating in hospitals and have been observed in outpatient settings as well (Chaudhary and Aggarwal, 2004). In the present study, in addition to being ESBL producing, all tested *E. coli* isolates were resistant to lomefloxacin as well as norfloxacin, and 14 out of the 17 isolates (82.4%) were resistant to trimethoprim-sulphamethoxazole. However, no resistance could be detected by the planktonically grown ESBL producing *E. coli* isolates towards nitrofurantoin. Our results are comparable

to the results of Auer et al. (2010) and Mukherjee et al. (2013). Also, Tasbakan et al. (2012) reported clinical and micro-biological success rates of 69% and 68%; respectively by nitrofurantoin on 75 patients with ESBL-producing *E. coli* related lower UTIs. A finding which they reported supports the suggestion that nitrofurantoin may be an alternative treatment for UTIs caused by such organisms.

Regarding isolates of the *Enterococcus* spp., our study detected resistance to nitrofurantoin in only one out of the 24 (4.2%) isolates. Comparable results have been reported by others (Chayakul et al., 2007; Karlowsky et al., 2011). On the other hand, higher resistance was detected towards other antibiotics; where 25% of the isolates were resistant to each of levofloxacin and norfloxacin, 29.2% were resistant to ciprofloxacin and 87.5% were resistant to tetracycline. Similar findings have been reported by Butt et al. (2004), who noticed that most of the nitrofurantoin susceptible enterococcal isolates were resistant to all other available antibiotics.

A wide range of microorganisms have been shown to be sensitive to crushed garlic preparations increasing the interest towards garlic as a medicinal panacea (Ankri and Mirelman, 1999). In the present study, garlic showed an inhibitory effect on ESBL producing *E. coli* with concentrations ranging from 6250 to 100,000 $\mu\text{g/ml}$ and a mean of $37,867 \pm 31,130.285$. Lower results have

been demonstrated for commensal and pathogenic *E. coli* isolates with garlic MIC ranging between 3,130 and 12,500 $\mu\text{g/ml}$. (Ross et al., 2001). On the other hand, almost comparable results have been shown towards multi-drug resistant *E. coli*, where a mean garlic MIC of $20,800 \pm 6,100$ was observed (Iwalokun et al., 2004). In addition, Abubakar (2009) reported garlic MIC values of 50,000 $\mu\text{g/ml}$ and 100,000 $\mu\text{g/ml}$ towards a standard laboratory *E. coli* strain and a nosocomial *E. coli* isolate, respectively. The current study also showed an inhibitory effect for garlic on *Enterococcus* spp. with a range of 25,000 to 75,000 $\mu\text{g/ml}$. Lower values have been reported by Jonkers et al. (1999) and Bokaeian and Bameri (2013); with a range of 4000 to 8000 $\mu\text{g/ml}$ and a range of 4000 to 32,000 $\mu\text{g/ml}$, respectively. The disparity of antimicrobial potency for garlic observed among studies might be attributed to the geographical variation which affects the intensity and range of antibacterial effects of garlic (Bokaeian and Bameri, 2013). Moreover, the concentrations of the effective components of garlic have been found to vary by its age and method of preparation (Iwalokun et al., 2004; Bokaeian and Bameri, 2013).

An important issue in the treatment of bacterial biofilm infections is the lowered effectiveness of antibiotics (Jakobsen et al., 2012), which arises from multiple mechanisms such as failure of the antibiotic to penetrate the full length of the biofilm

and the reduced bacterial growth rate among some of the cells due to nutrient limitation (Costerton et al., 1999). In the present study, the urinary isolates that were either sensitive or intermediately sensitive to nitrofurantoin when grown as planktonic cells became resistant in their sessile mode of growth. The nitrofurantoin MIC towards biofilm grown *Enterococcus* spp. exceeded 512 µg/ml and its MIC towards biofilm grown *E. coli* isolates rose between two to 32 times compared to their planktonic counterparts. In a study of urinary *E. coli* isolates, Ghanwate (2012) noticed an increase in the nitrofurantoin MIC 17 times from planktonic to sessile growth. It has also been stated that more than 100 times the MIC of antibiotics is required to eradicate cells within a biofilm (Sharma et al., 2009).

An important question in this research was the effect of garlic on already formed biofilms. Garlic MIC exceeded 800,000 µg/ml for the biofilm grown *Enterococcus* spp, and it increased between two to 32 times for the *E. coli* isolates when grown as biofilm. Although their study involved a different genus than ours, Shuford et al. (2005) reported that the *in vitro* activity of fresh garlic extract on a *Candida albicans* biofilm decreased as the biofilm phenotype developed from the early to the mature phase.

The present study set out to determine the effect of combining nitrofurantoin and garlic on the studied urinary isolates. There was a significant decline in the MIC values for each of nitrofurantoin and garlic in combination on the planktonic growth of all isolates compared to their MICs alone. The result of which was mostly synergy or addition, with more synergism in favour of ESBL producing *E. coli* compared to enterococci. These findings further support the idea that garlic combination with antibiotics holds promising effects. Synergism has been observed between garlic and vancomycin against vancomycin resistant enterococci (Jonkers et al., 1999). Garlic has also shown synergy with streptomycin against streptomycin resistant *E. coli* (Palaksha et al., 2010).

However, to the best of our knowledge, previous studies have not addressed the effect of the combination of garlic with antibiotics on already formed biofilms. The MICs for each of nitrofurantoin and garlic towards biofilm grown enterococcal isolates rose to high levels from which we could not expand our studies to test for their effect in combination. However, we were able to test their combined effect on the three biofilm forming ESBL producing *E. coli* isolates. Although their independent MICs on the formed biofilms rose to high levels, surprisingly, the MIC values of each of garlic and nitrofurantoin, when combined, dropped 4 times for garlic and between 2 to 128 times for nitrofurantoin; which resulted in either synergy or addition.

It seems possible that our results concerning combination between garlic and nitrofurantoin might be related to the quorum sensing blocking property of garlic (Bodini et al., 2009), which presumably allowed nitrofurantoin to act more efficiently. Our hypothesis is supported by the

work of Rasmussen et al. (2005), who demonstrated almost complete elimination of an established *Pseudomonas aeruginosa* biofilm grown in the presence of garlic extract after being treated by tobramycin, in contrast to an untreated biofilm, grown with garlic, which remained viable and a tobramycin treated biofilm, grown without garlic, where only cells in the top layer were killed.

In conclusion, it appears that combinations may be more useful than individual agents. Our results suggest a possible role for garlic in enhancing the antibacterial activity of nitrofurantoin against planktonic and sessile forms of growth. It is thus recommended that further research would be undertaken by utilizing either crude garlic extract or specifically identified molecules obtained from garlic, to be tested alone and in combination with nitrofurantoin towards more urinary isolates of different genera in planktonic and sessile forms before addressing pharmaceutical companies for future combinatory treatment in UTIs.

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