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Original article

Antimicrobial potential of unstressed and heat stressed *Allium sativum*

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ABSTRACT

Garlic (*Allium sativum*) is generally known to be of medicinal value, possessing potentials that include antimicrobial activity, but are often consumed in foods after subjection to cooking heat. The antimicrobial potential of heat stressed garlic may become decreased or lost when cooked, making its medicinal benefit unavailable to consumers. The potential of uncooked and cooked extracts from garlic imported to Jamaica, to inhibit the growth of eight microbes of clinical significance was investigated. Aqueous extracts of fresh garlic of 15 g/100 ml (fw), and dried and pulverized garlic cloves of 12.5 g/100 ml, 25 g/100 ml, 50 g/100 ml, and 100 g/100 ml (dw), were tested for inhibition of microbial growth. Extracts were tested uncooked, and cooked by boiling for 5, 10, and 15 min respectively. Of all the microbes studied, *C. albicans* incurred the largest zone of inhibition (57.7 ± 0.6 mm at the 100 g/100 ml of the dried extract, $F(3, 8) = 51.778$, $p < 0.001$, $\omega^2 = 0.93$). Cooking of garlic extracts resulted in statistically significant decreases in zones of inhibition of microbes, as evident in the linear regression and one-way ANOVA analyses, and/or complete loss of microbial inhibition. *C. albicans* was the most inhibited microbe, followed by *E. coli*, and *Salmonella* sp., respectively. The use of uncooked garlic may be the best route for obtaining the greatest antimicrobial potential of garlic against susceptible bacteria and fungi because cooking heat stress resulted in the decrease and complete loss of the antimicrobial potentials of the garlic.

1. Introduction

The *Allium* genus is believed to be one of the oldest horticultural crops in history. Allium is among the largest cultivated herbal plants and is known to be of much economic and folklore medicinal importance (El-Saber Batiha et al., 2020). Different species, including garlic, onions, leeks, shallots, etc. have been essentially used as ingredients in culinary practices to flavour foods. Several of these herbs and spices have been used in ethnomedicinal practices, to cure and alleviate ailments for many years. Garlic (*Allium sativum*) has been used for millennia as a flavouring for foods and meats and has migrated to the shelves of many households as a nutraceutical agent for treatment of several ailments. Garlic is rich in several sulphur-containing compounds of medicinal value beneficial to human health, and they include alliin (S-allyl L-cysteine sulfoxide), methiin (S-methylcysteine sulfoxide), isoalliin, and flavonoids which include quercetin (El-Saber Batiha et al., 2020; Tedeschi et al., 2022). Alliin is converted mostly to allicin (allyl 2-propenethiosulfinate), when it interacts with the enzyme alliinase, on crushing of the garlic clove. Allicin, which is sparingly soluble in water, is the active compound that gives garlic its characteristic odour and flavour. Garlic, its bioactive compounds, and aged garlic extract (AGE), are reported to have activities of pharmacological significance, which include antioxidant, anti-inflammatory, anticarcinogenic, antiplatelet, antithrombotic, antidiabetic, antibacterial, antifungal, antiviral, antiprotozoal, and hypocholesterolemic properties (Amagase et al., 2001; El-Saber Batiha et al., 2020; Tedeschi et al., 2022).

Since preparation of foods mostly involves the application of heat by cooking, such as boiling in water, to enable their safe consumption, garlic in these foods are usually subjected to heat before being consumed. However, a knowledge of the health benefits,

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including antimicrobial activities, that may be derived or lost from the garlic under such circumstances is important. In this study, the potential of uncooked and cooked *A. sativum* extracts from bulbs/-cloves, obtained from the Jamaican market, to inhibit named microbes of clinical significance was investigated. Such microbes include the fungus *Candida albicans*, which is a commensal that forms part of the normal flora of the microbiota in humans but is one of about 20 Candida species that cause infections in humans (Macias-Paz et al., 2023). It is the main causative agent of candidiasis and is reported to account for about 70% of fungal infections globally, with a mortality rate of about 40% for life-threatening invasive infections, especially in hospital conditions (Talapko et al., 2021).

Among the bacteria are *Escherichia coli* and *Klebsiella pneumoniae*, which belong to the Enterobacteriaceae family and are typically commensals that mainly colonize the colon of mammals, including humans. *E. coli* has notably been widely utilized for cloning in recombinant DNA technology. However, the *E. coli* species contains some pathogenic strains that prove to be virulent. It is also reported to be the most infective agent responsible for extraintestinal, urinary tract infections and one of the causative agents of meningitis and sepsis (Kaper et al., 2004). The intestinal pathogenic strains include the enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (Nataro & Kaper, 1998). *K. pneumoniae* is reported to be both a commensal and an opportunistic pathogen to humans. It is reported to be a leading cause of nosocomial infections and has strains of the species that are hypervirulent and others that are antibiotic-resistant (Li et al., 2022; Martin & Bachman, 2018).

2. Materials and methods

2.1. Sourcing of materials and equipment for extraction

Fresh *A. sativum* (garlic) bulbs that were commercially imported to Jamaica from China were purchased at the local supermarket in Mandeville, Jamaica in March 2018, and utilized for this study because garlic is widely consumed but currently not cultivated in Jamaica. They were stored at room temperature in a dry environment until used. The dried garlic samples were stored in the refrigerator at 2–8 °C after pulverization until utilized. All microbes that were utilized in this study were obtained from the Medical Technology Department laboratory at Northern Caribbean University, Mandeville, Jamaica. The Gourmia GFD 1680 food dehydrator and the NutriBullet NBR-1201 high speed blender were purchased from the USA. All extract preparation equipment were thoroughly washed and rinsed with sterile water prior to use.

2.2. Aqueous extraction of garlic

The garlic bulbs and cloves were macroscopically observed, and the freshly looking, non-speckled and robust ones were selected for use. They were washed with sterile distilled water and dried aseptically. Weighed garlic cloves were blended with the Vitamix Professional blender (Vitamix, Cleveland, OH, USA) in sterile distilled water for about 3–5 min until homogeneity of blended material was achieved, to make the fresh garlic extract of 15 g/100 ml (fw). The dried garlic extract was prepared by preliminarily slicing selected bulbs and cloves, and then subjecting them to drying using the Gourmia GFD1680 food dehydrator (Gourmia, Brooklyn, NY, USA) for 60 hr at 45 °C. The dry samples were pulverized into powder using the NutriBullet NBR-1201 high speed blender (Nutribullet, Los Angeles, CA, USA). The powder was used to prepare aqueous garlic extract suspensions of 12.5, 25, 50, and 100 g/100 ml (dw), respectively, with sterile distilled water. All dried garlic extract samples were incubated for 4 hr prior to application to agar plates for microbial inhibition testing.

2.3. Heating of garlic extracts

Fresh, and dried garlic extract samples of different concentrations were heat stressed by boiling in water as the means of cooking for 5, 10, and 15 min, respectively. They were then cooled to room temperature before application to wells for microbial inhibition testing.

2.4. Test for microbial inhibition

Eight clinically significant microbes were subcultured in agar plates at 37 °C for 24 hr and utilized for this study. They include the Gram-positive *Streptococcus pneumoniae*, Gram-negative *Klebsiella oxytoca*, *Pseudomonas* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* sp., and the fungus *Candida albicans*. The bacteria were grown using the Mueller Hinton agar, and the fungus, using the Sabouraud dextrose agar media. A colony suspension of each microorganism was prepared, with a suspension turbidity equivalent to 0.5 McFarland standard (about 1.5×10^8/ml) and used to inoculate the relevant agar plates, with the continuous streaking method. The plates were utilized for the agar well diffusion assay. A 6 mm diameter sterile cork borer was used to make wells on the agar plates. Aliquots of 50 µl of defined concentrations of unheated and heated fresh and dried garlic extracts were introduced into wells created in agar plates inoculated with the respective stated microbes. The plates were incubated at 37 °C for 24 hr and observed for susceptibility to antimicrobial activity. Susceptibility was examined through determination of the diameter of zone of inhibition of microbial growth. Sample studies were conducted in triplicates and the mean values of their measurements obtained. Agar plates of all eight microbes with no garlic extract added served as negative controls. The antibiotics gentamycin (10 µg) and chloramphenicol (30 µg) discs were applied to agar plates of all bacteria except *S. pneumoniae*, and the antifungal agent fluconazole (25 µg) discs were applied to *C. albicans* agar plates, to serve as positive controls for zones of inhibition, for all garlic extract concentrations, respectively.

2.5. Statistical analysis

Standard deviations from the means of zones of inhibition incurred were calculated for all microbes affected by any garlic extract sample. The statistical significance of the effect of concentration, or length of time of heat stress by cooking, of the garlic extract, on the zone of inhibition of each microorganism was determined using the one-way analysis of variance (ANOVA), and linear regression, and the combination thereof by the two-way ANOVA. Probability values (p) < 0.05 were considered statistically significant. Statistical analyses were carried out using the SPSS software, version 28.

3. Results

The antimicrobial abilities of the different concentrations of garlic extracts, which included fresh and dried garlic respectively, were obtained by conduction of the microbial growth inhibition tests of the garlic extracts on eight microbes listed in section 2.4 above.

3.1. Uncooked and cooked fresh garlic extract

Fig. 1 shows that *S. pneumoniae* and *Pseudomonas* sp. were not susceptible to the antimicrobial activity of uncooked and cooked fresh garlic extract of 15 g/100 ml. *C. albicans* incurred the highest zone of inhibition of 30.0 ± 0.0 mm from the uncooked garlic, and 24.7 ± 2.3 mm from the cooked up to 5 min. It however suffered no zone of inhibition from the extract that was heat stressed by cooking at the boiling temperature of 98 °C for 10 min. *E. coli*, *P. mirabilis*, and *Salmonella* sp. experienced zones of inhibition, which decreased with increase in extract cooking time, so that they had relatively mild inhibition of 11.0 ± 1.0,12.7 ± 0.6, and 12.0 ± 0.0 m m, respectively at 10 min of cooking. A linear regression established that cooking time up to 10 min could statistically significantly predict the zones of inhibition of *E. coli*, $F(1, 7) = 131.250$, $p < 0.001$, and *P. mirabilis*, $F(1, 7) = 79.154$, $p < 0.001$. Such could not be established for the *Salmonella* sp. because it violated the Durbin-Watson statistic, with a value of 1.319. Within this cooking time, the prediction equation for *E. coli* was zone of inhibition (mm) = 21.000 + (−1.000 × (extract cooking time in min)); for *P. mirabilis*, it was: zone of inhibition (mm) = 19.833 + (−0.700 × (ex tract cooking time in min)). No zone of inhibition was obtained for any of the microbes when the extract was heat stressed by cooking at 98 °C for up to 15 min.

3.2. Uncooked dried garlic extract

The uncooked dried garlic extract generally resulted in increase in zone of inhibition with concentration, for seven of the eight microbes, but *K. pneumoniae* experienced no zone of inhibition (Figs. 2–5). A one-way ANOVA conducted to determine if the zones of inhibition experienced by each inhibited microbe were different among the four concentrations, showed that there were no outliers, as assessed by boxplot. There was homogeneity of variances for five of the seven microbes, as assessed by Levene's test of homogeneity of variances. They were *C. albicans* ($p = 0.057$), *S. pneumoniae* ($p = 0.222$), *Salmonella* sp. ($p = 0.271$), *E. coli* ($p = 0.292$), and *K. oxytoca* ($p = 0.367$). *Pseudomonas* sp. was not considered to pass the homogeneity of variances test ($p = 0.050$), and *P. mirabilis* violated this test ($p = 0.030$). Zones of inhibition were significantly different among different concentrations of the uncooked dried garlic extract for the five stated microbes above (see Table 1). The statistical significance values were $F(3, 8) = 18.658$, $p < 0.001$, $\omega^2 = 0.82$ for *Salmonella* sp., $F(3, 8) = 33.507$, $p < 0.001$, $\omega^2 = 0.89$ for *S. pneumoniae*, $F(3, 8) = 44.991$, $p < 0.001$, $\omega^2 = 0.92$ for *K. oxytoca*, $F(3, 8) = 51.778$, $p < 0.001$, $\omega^2 = 0.93$ for *C. albicans*, and $F(3, 8) = 88.048$, $p < 0.001$, $\omega^2 = 0.96$ for *E. coli*. Data from Table 1 and Figs. 2–5 indicate that for *S. pneumoniae*, zone of inhibition increased from 14. 7 ± 0.6 mm at extract concentration of 12.5 g/100 ml, to 15.3 ± 0. 6 mm at 25 g/100 ml, to 23.0 ± 1.7 mm at 50 g/100 ml, and to 23.0 ± 2.0 mm at 100 g/100 ml. Tukey's post hoc analysis revealed that the increase in zone of inhibition obtained from 12.5 g/100 ml to that at 50 g/100 ml and from 12.5 g/100 ml to 100 g/100 ml were the same (8.33 mm, 95% CI (4.71 mm to 11.95 mm)) and were both statistically significant ($p < 0.001$). The increase from 25 g/100 ml to 50 g/100 ml and from 25 g/100 ml to 100 g/100 ml were also the same (7.67 mm, 95% CI (4.05 mm to 11.29 mm), $p < 0.001$), and were each also statistically significant. However, the increases in microbial inhibition obtained from the zone of inhibition at 12.5 g/100 ml to that at 25 g/100 ml, and from 50 g/100 ml to 100 g/100 ml, were not statistically significant. This trend was similarly observed for *E. coli*, *Salmonella* sp., and *C. albicans*. The post hoc analysis for *K. oxytoca* showed that all increases of zone of inhibition with increase in extract concentration were statistically significant, except only for that from 50 g/100 ml to 100 g/100 ml.

A linear relationship of the effect of concentration of the dried garlic extract on zone of inhibition could not be established for all susceptible microbes. Independence of residuals could not be established as assessed by the Durbin-Watson statistic, which were mostly outside the range of 1.500 to 2.500. Also, the two-way ANOVA analysis indicates that a statistically significant interaction could not be established between the effects of concentration and cooking time of the dried garlic extract on the zone of inhibition of the susceptible microbes.

3.3. Effect of cooking of the dried garlic extract

Only *C. albicans* and *E. coli* consistently showed inhibitions up to 15 min of cooking at all concentrations of the dried extract, trending a decrease with increase in cooking time at 12.5, 25.0 and

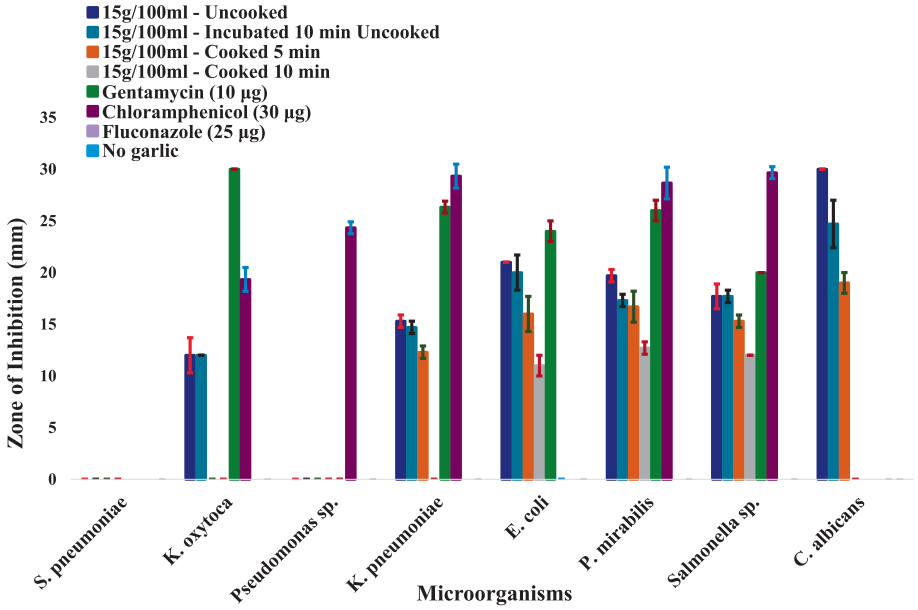

Fig. 1. Antimicrobial Activity of Uncooked and Cooked Fresh Garlic Aqueous Extract (15 g/100 ml).

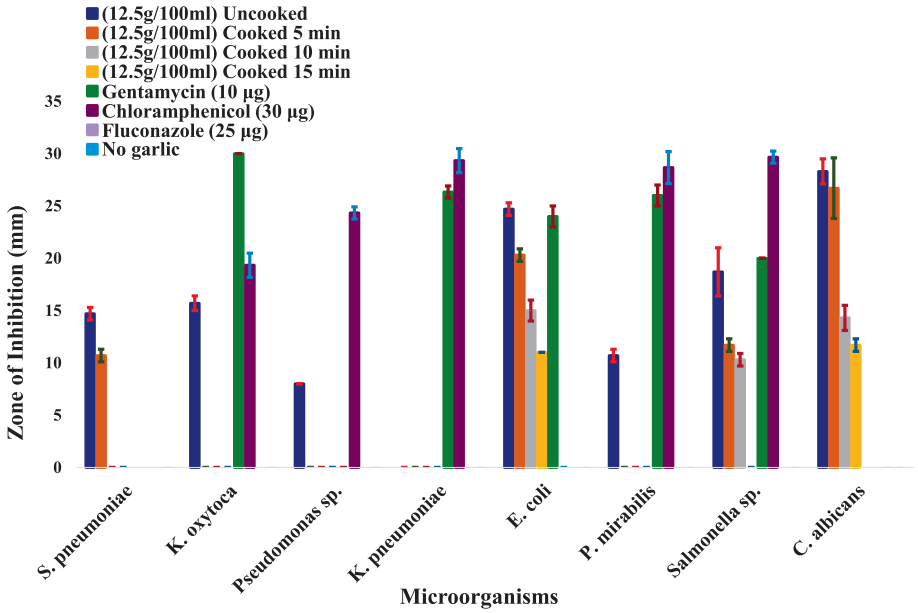

Fig. 2. Antimicrobial Activity of Uncooked and Cooked Dried Garlic Aqueous Extract (12.5 g/100 ml).

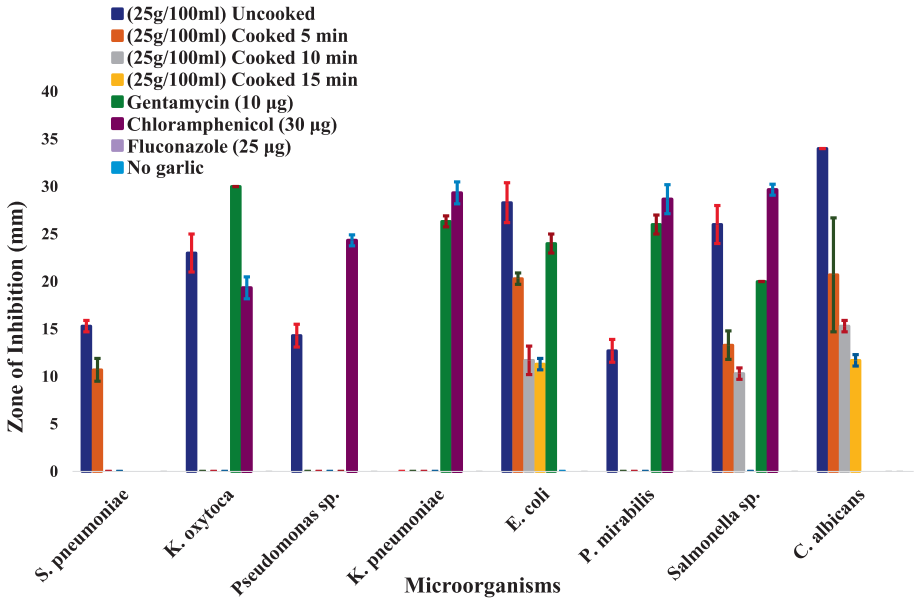

Fig. 3. Antimicrobial Activity of Uncooked and Cooked Dried Garlic Aqueous Extract (25 g/100 ml).

100 g/100 ml. *Salmonella* sp. showed a trend of decreasing inhibition up to 10 min of extract cooking, except for the 50 g/100 ml extract which also caused inhibition at 15 min of heat stress (Figs. 2–5).

The dried extract was seen to produce zones of inhibition on the growth of *Pseudomonas* sp., only when uncooked, at 12.5 g/100 ml and 25 g/100 ml, but also caused mild zones of inhibition when heat stressed for 5 min at 50 and 100 g/100 ml. The extract was observed to effectuate inhibition on *K. oxytoca* and *P. mirabilis* only when uncooked, at all four concentrations (Figs. 2–5). However, *K. oxytoca* had significantly larger zones of inhibition than the latter. It is noteworthy that *K. pneumoniae* was apparently not susceptible to both the unstressed and heat stressed dried extract at all the concentrations.

4. Discussion

Garlic has been widely reported in history to perform antibacterial and antifungal functions among other activities in ethnomedicinal practices and modern research studies. The eight microorganisms of clinical significance utilized in this study by reason of frequency of their implications in human infections, displayed different susceptibility responses to the unstressed and heat stressed fresh and dried garlic extracts (Figs. 2–5). *K. pneumoniae* exhibited resistance to the inhibitory activity of the dried garlic extract at all four concentrations tested. In contrast, this microbe was inhibited by the uncooked fresh garlic extract of 15 g/100 ml to a zone length of 15.3 ± 0.6 mm. The inhibition was attenuated by heat stress on the extract, which registered a

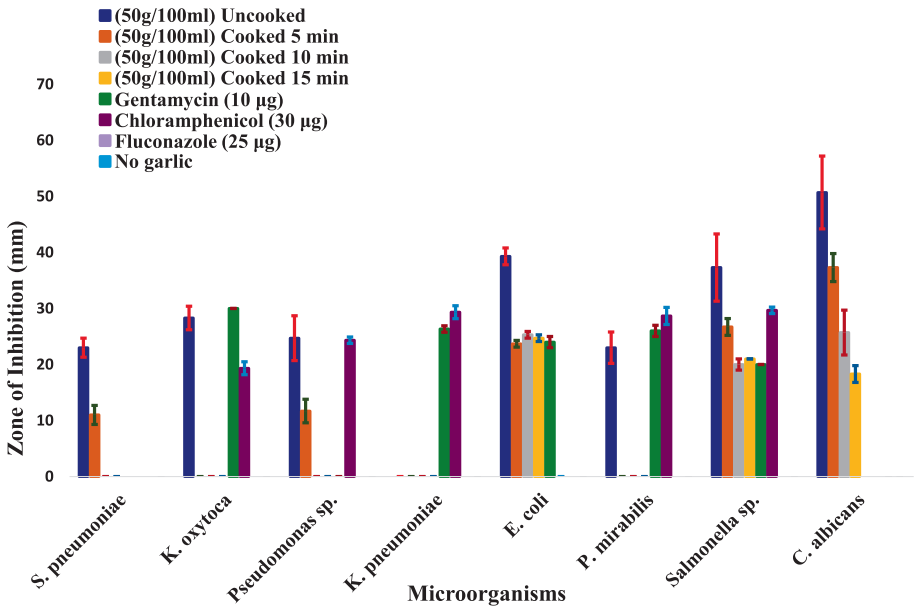

Fig. 4. Antimicrobial Activity of Uncooked and Cooked Dried Garlic Aqueous Extract (50 g/100 ml).

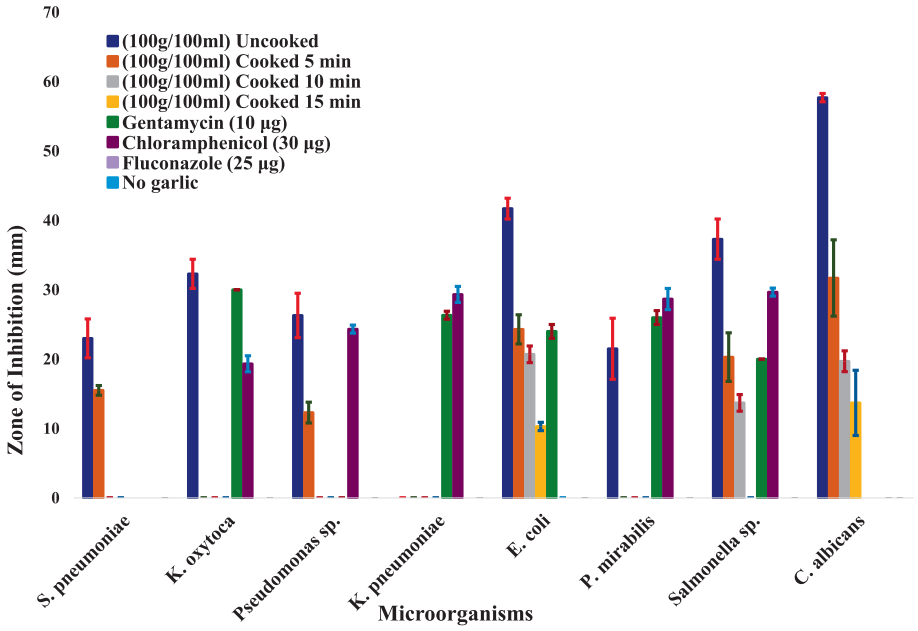

Fig. 5. Antimicrobial Activity of Uncooked and Cooked Dried Garlic Aqueous Extract (100 g/100 ml).

zone length of 12.3 ± 0.6 mm when cooked for 5 min (Fig. 1). The result may be suggesting that persons exposed to the hazards of *K. pneumoniae* could possibly only benefit from consumption of uncooked fresh garlic, rather than dried garlic, for protection against infection. *K. pneumoniae* is among the normal enterobacteria in humans but may become infective to persons if it enters other parts of the body, causing diseases, which include urinary tract infections (UTIs), pneumonia, and other clinically significant infections. It has been reported to be a frequent source of hospital-acquired and community-acquired UTIs, with a possible profile of increasing antibiotic resistance (Caneiras et al., 2019). The seven microorganisms inhibited by the uncooked dried garlic extract maintained a general trend of increase in zones of inhibition with extract concentration (see Figs. 2–5). The differences in the mean zones of inhibition at the different concentrations of the extract were demonstrated by the one-way ANOVA to be statistically significant for five of the inhibited microbes, which include *E. coli*. *K. oxytoca*, *S. pneumoniae*, *Salmonella* sp., and *C. albicans* (please, see results in section 3.2 above). However, consequent upon the lack of consistency in increase in lengths of zones of inhibition with increase in garlic extract concentration, a linear relationship could not be established between the concentration of the uncooked dried garlic extract and the zone of inhibition produced on the affected microbes. This suggests that the effect of increase in concentration of dried garlic extract on enhancement of its antimicrobial activity against susceptible microbes may follow a pattern that is not of linear progression. It may therefore be beneficial to determine the range of concentrations of dried garlic extract that would result in its optimal activity against the susceptible microbes.

Table 1
Significance of increase in inhibition of susceptible microbes with concentration (between 12.5 g/100 ml and 100 g/100 ml) of uncooked dried garlic extract.

Microbe	Garlic extract concentration (g/100 ml)	Mean Zone of Inhibition (mm)	Std. Deviation (mm)	Analysis of variance (ANOVA) ($F(3, 8)$)	ANOVA p-value*	Effect size (ω^2)	Homogeneity of variances: Levene's statistic** and sig. (p)	Increase in inhibition (mean difference (mm))	Tukey's HSD+* post hoc test significance (p)	Whether increase is statistically significant?
C. albicans	12.5	(a) 28.3	1.2	51.778	<0.001	0.93	3.847 (0.057)	(b)-(a) = 5.7	0.234	No
	25	(b) 34.0	0.0					(c)-(a) = 22.4	< 0.001	Yes
	50	(c) 50.7	6.5					(d)-(a) = 29.4	< 0.001	Yes
	100	(d) 57.7	0.6					(c)-(b) = 16.7	0.001	Yes
								(d)-(b) = 23.7	< 0.001	Yes
								(d)-(c) = 7.0	0.119	No
E. coli	12.5	(e) 24.7	0.6	88.048	<0.001	0.96	1.478 (0.292)	(f)-(e) = 3.7	0.072	No
	25	(f) 28.3	2.1					(g)-(e) = 14.7	< 0.001	Yes
	50	(g) 39.3	1.5					(h)-(e) = 17.0	< 0.001	Yes
	100	(h) 41.7	1.5					(g)-(f) = 11.0	< 0.001	Yes
								(h)-(f) = 13.3	< 0.001	Yes
								(h)-(g) = 2.3	0.311	No
K. oxytoca	12.5	(i) 15.7	0.6	44.991	<0.001	0.92	1.209 (0.367)	(j)-(i) = 7.3	0.005	Yes
	25	(j) 23.0	2.0					(k)-(i) = 12.6	< 0.001	Yes
	50	(k) 28.3	2.1					(l)-(i) = 16.0	< 0.001	Yes
	100	(l) 31.7	2.1					(k)-(j) = 5.3	0.028	Yes
								(l)-(j) = 8.7	0.002	Yes
								(l)-(k) = 3.4	0.186	No
***Salmonella* sp.**	12.5	(m) 18.7	2.3	18.658	<0.001	0.82	1.567 (0.271)	(n)-(m) = 7.3	0.145	No
	25	(n) 26.0	2.0					(p)-(m) = 18.7	0.001	Yes
	50	(p) 37.3	6.0					(q)-(m) = 18.7	0.001	Yes
	100	(q) 37.3	2.9					(p)-(n) = 11.3	0.022	Yes
								(q)-(n) = 11.3	0.022	Yes
								(q)-(p) = 0.0	1.000	No
S. pneumoniae	12.5	(r) 14.7	0.6	33.507	<0.001	0.89	1.816 (0.222)	(s)-(r) = 0.7	0.932	No
	25	(s) 15.3	0.6					(t)-(r) = 8.3	< 0.001	Yes
	50	(t) 23.0	1.7					(u)-(r) = 8.3	< 0.001	Yes
	100	(u) 23.0	2.0					(t)-(s) = 7.7	< 0.001	Yes
								(u)-(s) = 7.7	< 0.001	Yes
								(u)-(t) = 0.0	1.000	No

* = at 95% confidence interval.
** = based on mean.
+* = Honest Significant Difference.

A prior incubation of the fresh garlic extract for 10 min before application to the inoculated agar plate wells generally did not enhance the zone of inhibition of the six inhibited microbes (Fig. 1). On the surface, this is seeming to suggest that once crushed, there may be no need to wait for up to 10 min to derive the antibiotic benefit from garlic. However, further analysis may be necessary to tell the mechanistic process of reactions and changes occurring in crushed garlic and the roles of the various components in resulting in antimicrobial inhibition. Garlic is known to be rich in sulphur compounds and the process of crushing causes reactions that include the interaction of alliin with the enzyme alliinase to form allicin. Allicin, which has been suspected to be one of the main compounds that may be responsible for antimicrobial activity of garlic, is reported to be relatively unstable and would further lead to the derivation of other metabolites which include ajoene, diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide (DATS), etc. (Fujisawa et al., 2008; Iciek et al., 2009; Salehi et al., 2019; Tedeschi et al., 2022). However, the roles and mechanism of each of these compounds or a combination thereof in causing inhibition of microbial growth are yet to be established and need to be explored.

Only the uncooked extracts of both fresh garlic of 15 g/100 ml and dried garlic of all concentrations produced zones of inhibition of *K. oxytoca*. While extract cooking led to the apparent elimination of susceptibility of *P. mirabilis* to inhibition by the dried garlic extract at all four concentrations, the fresh garlic extract was observed to produce inhibition up to 10 min of cooking. For the *Pseudomonas* sp., cooking of the dried garlic extract for 5 min at best significantly decreased the lengths of its zones of inhibition accomplished by the uncooked extracts of 50 g/100 ml and 100 g/100 ml, by over 50% respectively (Figs. 2–5). These results seem to suggest that uncooked dried garlic of no<50 g/100 ml concentration may be necessary to produce a significant susceptibility of *Pseudomonas* sp. whereas fresh garlic extract may be the better option for obtaining the susceptibility of *P. mirabilis*. The susceptibility profile of *K. oxytoca* seems to propose that only the uncooked garlic extract, whether fresh or dried, could result in its inhibition, and that the higher the concentration of the uncooked extract is the greater its susceptibility to inhibition, $F(3, 8) = 44.991$, $p < 0.001$, $\omega^2 = 0.92$. Although the Gram-positive bacterium, *S. pneumoniae*, also known as pneumococcus, showed some level of susceptibility to the antimicrobial activity of the dried garlic extract at all concentrations examined, it was resistant to inhibition by the fresh extract of 15 g/100 ml. The dried extract cooked for 5 min produced its reduced zones of inhibition at all the concentrations, but not with cooking for 10 min and beyond. This seems to suggest that the dried garlic extract, especially when uncooked, might be the means of pursuit of inhibition of *S. pneumoniae* rather than the fresh. There are over 100 serotypes of *S. pneumoniae* colonizing the mucosa of the upper respiratory tract, most of which can cause disease but only a few are responsible for most of the pneumococcal infections in humans (Weiser et al., 2018). It is one of the 12 priority pathogens in the listing of the World Health Organization (WHO) by reason of its persistent high burden of disease and rising rates of resistance to common antibiotics, including penicillin (Weiser et al., 2018). Zhu and Zeng (2020) have reported the ability of fresh garlic extract to demonstrate antibiotic and antibiofilm activity against Gram-positive and Gram-negative bacteria, including multidrug resistant bacteria, and against fungi. However, it is needful from the findings of this study, to explore similar studies with the dried garlic extract for comparative analysis of relative efficacy.

The susceptibility of *C. albicans* to the antimicrobial activity of the uncooked extract, demonstrating it as the most significantly inhibited microbe, was evident across all concentrations, ranging from 28.3 ± 1.2 mm zone of inhibition at 12.5 g/100 ml to 57.7 ± 0.6 mm at the 100 g/100 ml of the dried extract, $F(3, 8) = 51.778$, $p < 0.001$, $\omega^2 = 0.93$. Although the fresh extract appeared to have lost its *C. albicans* inhibition activity when cooked for 10 min, the dried extract seemed to produce a greater level of sustained but decreasing inhibition of the fungus under heat stress subjugation. *C. albicans* is one of the *Candida* species said to be responsible for most of the infections caused by fungal pathogens and is itself the most frequent fungal cause of opportunistic infections in humans (Lopes & Lionakis, 2022). *C. albicans* is a commensal yeast fungus that inhabits the human oral, vaginal, and gastrointestinal mucosal tracts, and skin, asymptomatically (Beigi et al., 2004; Bougnoux et al., 2006; Lopes & Lionakis, 2022). Its opportunistic pathogenesis could cause debilitating mucocutaneous disease and/or life-threatening systemic infections on disturbance of the host barrier integrity or when the host becomes immunocompromised (Lopes & Lionakis, 2022). The results in this study suggest that the uncooked garlic extracts, especially the dried at higher concentrations, show great promise in preventing and/or managing such infections. This suggestion is even more pronounced given the fact that the fungus was found to be resistant to the antifungal compound, fluconazole (25 μg), used as a positive control in this study. The potential of the extracts for yeast inhibition may still be observed, but however significantly decreased, if cooked for no more than 5 min.

Among the bacteria tested for antimicrobial activity, *E. coli* was the most significantly susceptible to the uncooked and cooked garlic extracts at all concentrations examined. While the uncooked dried extract gave significant increasing zones of inhibition of *E. coli* from 24.7 ± 0.6 mm at 12.5 g/100 ml to 41.7 ± 1.5 mm at 100 g/100 ml, $F(3, 8) = 88.048$, $p < 0.001$, $\omega^2 = 0.96$, the antimicrobial activity of the cooked extract was generally seen to be decreasing with increase in cooking time (Figs. 2, 3 & 5). Linear regression established that cooking time of garlic extract could statistically significantly predict the zone of inhibition of *E. coli* by the fresh extract, up to 10 min of cooking, $F(1, 7) = 131.250$, $p < 0.001$. There was independence of residuals, as assessed by a Durbin-Watson statistic of 1.875. Cooking time for the dried extract could also predict the zone of inhibition of this microbe, up to 15 min of cooking, at 12.5 g/100 ml, $F(1,10) = 760.669$, $p < 0.001$. Independence of residuals was established by a Durbin-Watson statistic of 2.394. At 25 g/100 ml of the dried extract, cooking time could statistically significantly predict the zone of inhibition of *E. coli*, up to 10 min of cooking, $F(1, 7) = 205.078$, $p < 0.001$. Independence of residuals was demonstrated by a Durbin-Watson statistic of 2.266. The results seem to suggest that garlic extract, especially when uncooked, dried, and made into aqueous suspension at concentrations up to 50 g/100 ml, may be effective in preventing and/or managing *E. coli* infection. This is particularly significant because although some *E. coli* strains are very common commensals to humans, some pathogenic strains, which include the Shiga toxin-producing *E. coli* (STEC), are among the pathogens that have been reported as the main causes of food poisoning and foodborne illnesses globally, with antibiotic-resistance profiles (Foley et al., 2013; Jang et al., 2017; Lee & Yoon, 2021; World Health Organization (WHO), 2018). Disease outbreaks from pathogenic *E. coli* infections usually arise from raw and undercooked animal protein sources such as unpasteurized milk and ground meat products, and from faecal contaminations of raw vegetables (WHO, 2018). The infection is in most cases self-resolving, but could sometimes be life-threatening, causing diseases like the haemolytic uraemic syndrome (HUS), especially among young children, the elderly, and the immunocompromised (Lee & Yoon, 2021; WHO, 2018). Significant outbreaks have been reported in different parts of the world, among which was the outbreak in Germany in 2011, caused by the enterohemorrhagic *E. coli* (EHEC) 0104:H4 serotype – a type of STEC. Out of the total of 3842 persons reportedly

infected in Germany as attributable to the outbreak, most of whom were adults, 2987 presented with gastroenteritis (without development of HUS), 855 developed HUS, and 53 persons died (Robert Koch Institute (RKI), 2011). The outbreak spread to 16 countries, including France, and the United States of America, with a final total of 4,075 cases (Foley et al., 2013). Pathogenic *E. coli* has been reported to be the highest cause of food poisoning in South Korea for 11 out of 18 years, between 2003 and 2020, with infections at a peak, affecting up to 2,754 persons in 2016 (Ministry of Food and Drug Safety (MFDS), 2022). Studies on the potential benefits of consumption of fresh and dried garlic extracts for prevention and/or management of pathogenic *E. coli* infections may become necessary for mitigation of antibiotic resistance by these pathogens. The studies are deemed pertinent, especially as *E. coli* was resistant to the antibiotic, chloramphenicol (30 μg), one of the positive controls, in this study.

Both fresh and dried garlic extracts produced antimicrobial activity against the *Salmonella* sp., with zones of inhibition inflicted by the uncooked extracts ranging from 17.7 ± 1.2 mm to 37.3 ± 2. 9 mm. The inhibition profile of the extracts shows that the antimicrobial activity of garlic extract against the *Salmonella* sp. characteristically decreases with cooking, and generally becomes lost after cooking beyond 10 min. The result seems to suggest that uncooked garlic extract, whether fresh or dried, should be more deeply studied for possible recommendation for prevention and/or management of *Salmonella* infection. This is deemed of valid importance as *Salmonella* has a reputation for causing food poisoning and foodborne illnesses, some of which may be markedly debilitating and are sometimes fatal. Infections by *Salmonella*, referred to as salmonellosis, has been one of the most common foodborne infections in humans for many decades. It remains a public health concern affecting the industrialized and developing nations, by reason of the relatively high infection rates and outbreaks that are reported to still be occurring by it in the contemporary world (Eng et al., 2015; Popa & Papa, 2021). Major salmonellosis is caused by *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi), and serovar Paratyphi (*S.* Paratyphi), which result in enteric fever symptoms that include typhoid fever and paratyphoid fever, respectively (Eng et al., 2015; Johnson et al., 2018; Popa & Papa, 2021; Qamar et al., 2020). They are often characterized by relatively high incidences of antimicrobial resistance and have resulted in fears of significant multidrug resistance (Gibani et al., 2018; Qamar et al., 2020). Minor salmonellosis, on the other hand, is infection caused by the nontyphoid *Salmonella enterica* serotypes, which usually leads to diarrhoea that is often self-limiting but could sometimes result in occurrences of hospitalizations, and deaths. For example, nontyphoidal *Salmonella* spp. caused the most hospitalizations, and the most deaths, among an estimated 9.4 million cases of foodborne illnesses elicited by infections from different pathogens in USA in 2011 (Scallan et al., 2011). Of this total, nontyphoidal *Salmonella* spp. infected over one million persons and resulted in an estimated 23,128 hospitalizations and 452 deaths. Contaminated foods which include eggs, meats (including poultry, pork, and beef), and dairy products are the most common sources of nontyphoidal salmonellosis, with eggs topping the list, while infections are also reported from contaminated nuts, infant formulas, raw vegetables, fruits, and water (Brenner et al., 2000; Ehuwa et al., 2021; Ford et al., 2016; Pires et al., 2014; Popa & Papa, 2021).

5. Conclusion

This study reveals that the uncooked garlic extract possesses significant ability to inhibit the growth of some microbes of clinical significance, which include the fungus *C. albicans*, Gram-negative bacteria *E. coli*, and *Salmonella* sp., and to a lesser extent, Gram-positive *S. pneumoniae*. It seems to have a huge potential for preventing and/or managing infections that may be caused by these microbes, for benefit to humans. The antimicrobial potential of the uncooked dried extract was observed to significantly increase with concentration for five of the microbes studied, as assessed by the one-way ANOVA analysis. The study also shows the limiting effect of heat stress on the antimicrobial ability of garlic extract. While the inhibition of *K. oxytoca* by garlic extract was lost due to cooking for as short as 5 min, *C. albicans*, *E. coli*, and *Salmonella* sp. still incurred susceptibility to heat stressed extracts, with trends of decreasing inhibitions up to 10 min of extract cooking. Cooking for up to 15 min at best showed minimal and insignificant zones of inhibition of the latter three microorganisms. From the standpoint of these findings, it may be recommended that cooking of garlic should be avoided, when intended for use for a possible fight against infection by any of the microbes demonstrated in this study to be susceptible to garlic extract. A wider range of modulations of the uncooked and cooked fresh and dried garlic extracts, and the mechanisms of their antimicrobial activities should be engaged in further studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Amagase, H., Petesch, B.L., Matsuura, H., Kasuga, S., Itakura, Y., 2001. Intake of garlic and its bioactive components. J. Nutr. 131 (3), 955S–962S. https://doi.org/10.1093/jn/131.3.955S.

Beigi, R.H., Meyn, L.A., Moore, D.M., Krohn, M.A., Hillier, S.L., 2004. Vaginal yeast colonization in nonpregnant women: a longitudinal study. Obstetrics Gynecol. 104 (5, Part 1), 926–930. https://doi.org/10.1097/01.AOG.0000140687.51048.73.

Bougnoux, M.-E., Diogo, D., François, N., Sendid, B., Veirmeire, S., Colombel, J.F., Bouchier, C., van Kruiningen, H., d'Enfert, C., Poulain, D., 2006. Multilocus sequence typing reveals intrafamilial transmission and microevolutions of Candida albicans isolates from the human digestive tract. J. Clin. Microbiol. 44 (5), 1810–1820. https://doi.org/10.1128/JCM.44.5.1810-1820.2006.

Brenner, F.W., Villar, R.G., Angulo, F.J., Tauxe, R., Swaminathan, B., 2000. Salmonella nomenclature. J. Clin. Microbiol. 38 (7), 2465–2467. https://doi.org/10.1128/JCM.38.7.2465-2467.2000.

Caneiras, C., Lito, L., Melo-Cristino, J., Duarte, A., 2019. Community- and hospital-acquired Klebsiella pneumoniae urinary tract infections in Portugal: virulence and antibiotic resistance. Microorganisms 7 (5). https://doi.org/10.3390/microorganisms7050138.

Ehuwa, O., Jaiswal, A.K., Jaiswal, S., 2021. Salmonella, food safety and food handling practices. Foods (Basel, Switzerland) 10. https://doi.org/10.3390/foods10050907.

El-Saber Batiha, G., Magdy Beshbishy, A., Wasef, L.G., Elewa, Y.H.A., Al-Sagan, A.A., Abd El-Hack, M.E., Taha, A.E., Abd-Elhakim, Y.M., Prasad Devkota, H., 2020. Chemical constituents and pharmacological activities of garlic (Allium sativum L.): a review. Nutrients 12 (3), 872.

Eng, S.-K., Pusparajah, P., Ab Mutalib, N.-S., Ser, H.-L., Chan, K.-G., Lee, L.-H., 2015. *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. Front. Life Sci. 8 (3), 284–293. https://doi.org/10.1080/21553769.2015.1051243.

Foley, C., Harvey, E., Bidol, S. A., Henderson, T., Njord, R., DeSalvo, T., Haupt, T., Mba-Jonas, A., Bailey, C., Bopp, C., Bosch, S. A., Gerner-Smidt, P., Mody, R. K., Nguyen, T.-A., Strockbine, N., Tauxe, R.V., 2013. Outbreak of Escherichia coli O104:H4 Infections Associated with Sprout Consumption — Europe and North America, May–July 2011. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6250a3.htm.

Ford, L., Glass, K., Veitch, M., Wardell, R., Polkinghorne, B., Dobbins, T., Lal, A., Kirk, M.D., 2016. Increasing Incidence of Salmonella in Australia, 2000–2013. PloS One 11, (10). https://doi.org/10.1371/journal.pone.0163989 e0163989.

Fujisawa, H., Suma, K., Origuchi, K., Seki, T., Ariga, T., 2008. Thermostability of Allicin determined by chemical and biological assays. Biosci. Biotech. Bioch. 72 (11), 2877–2883. https://doi.org/10.1271/bbb.80381.

Gibani, M.M., Britto, C., Pollard, A.J., 2018. Typhoid and paratyphoid fever: a call to action. Curr. Opin. Infect. Dis. 31 (5), 440–448. https://doi.org/10.1097/QCO.0000000000000479.

Iciek, M., Kwiecień, I., Włodek, L., 2009. Biological properties of garlic and garlic-derived organosulfur compounds. Environ. Mol. Mutagen. 50 (3), 247–265. https://doi.org/10.1002/em.20474.

Jang, J., Hur, H.-G., Sadowsky, M.J., Byappanahalli, M.N., Yan, T., Ishii, S., 2017. Environmental *Escherichia coli*: ecology and public health implications-a review. J. Appl. Microbiol. 123 (3), 570–581. https://doi.org/10.1111/jam.13468.

Johnson, R., Mylona, E., Frankel, G., 2018. Typhoidal Salmonella: Distinctive virulence factors and pathogenesis. Cell. Microbiol. 20 (9), e12939.

Kaper, J.B., Nataro, J.P., Mobley, H.L.T., 2004. Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2 (2), 123–140. https://doi.org/10.1038/nrmicro818.

Lee, H., Yoon, Y., 2021. Etiological agents implicated in foodborne illness world wide. Food Sci. Animal Resources 41 (1), 1–7. https://doi.org/10.5851/kosfa.2020.e75.

Li, Y., Kumar, S., Zhang, L., Wu, H., Kaushik, S., 2022. Klebsiella pneumonia and its antibiotic resistance: a bibliometric analysis. Biomed Res. Int. 2022, 1–10.

Lopes, J.P., Lionakis, M.S., 2022. Pathogenesis and virulence of *Candida albicans*. Virulence 13 (1), 89–121. https://doi.org/10.1080/21505594.2021.2019950.

Macias-Paz, I.U., Pérez-Hernández, S., Tavera-Tapia, A., Luna-Arias, J.P., Guerra-Cárdenas, J.E., Reyna-Beltrán, E., 2023. Candida albicans the main opportunistic pathogenic fungus in humans. Rev. Argent. Microbiol. 55 (2), 189–198. https://doi.org/10.1016/j.ram.2022.08.003.

Martin, R.M., Bachman, M.A., 2018. Colonization, Infection, and the Accessory Genome of Klebsiella pneumoniae. Front. Cell. Infect. Microbiol. 8, 4. https://doi.org/10.3389/fcimb.2018.00004.

Ministry of Food and Drug Safety (MFDS), 2022. Food Poisoning Statistics. https://www.foodsafetykorea.go.kr/portal/healthyfoodlife/foodPoisoningStat.do?menu_no=3724&menu_grp=MENU_NEW02.

Nataro, J.P., Kaper, J.B., 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11 (2), 403. https://doi.org/10.1128/CMR.11.2.403.

Pires, S.M., Vieira, A.R., Hald, T., Cole, D., 2014. Source attribution of human salmonellosis: an overview of methods and estimates. Foodborne Pathog. Dis. 11 (9), 667–676. https://doi.org/10.1089/fpd.2014.1744.

Popa, G.L., Papa, M.I., 2021. Salmonella spp. infection - a continuous threat worldwide. Germs 11 (1), 88–96. https://doi.org/10.18683/germs.2021.1244.

Qamar, F.N., Yousafzai, M.T., Dehraj, I.F., Shakoor, S., Irfan, S., Hotwani, A., Hunzai, M. J., Thobani, R.S., Rahman, N., Mehmood, J., Hemlock, C., Memon, A.M., Andrews, J.R., Luby, S.P., Garrett, D.O., Longley, A.T., Date, K., Saha, S.K., 2020. Antimicrobial resistance in typhoidal salmonella: surveillance for enteric fever in Asia project, 2016–2019. Clin. Infect. Dis. 71 (Supplement_3), S276–S284. https://doi.org/10.1093/cid/ciaa1323.

Robert Koch Institute (RKI), 2011. Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 Outbreak Germany 2011. https://www.rki.de/EN/Content/infections/epidemiology/outbreaks/EHEC_O104/EHEC_final_report.html.

Salehi, B., Zucca, P., Orhan, I.E., Azzini, E., Adetunji, C.O., Mohammed, S.A., Banerjee, S.K., Sharopov, F., Rigano, D., Sharifi-Rad, J., Armstrong, L., Martorell, M., Sureda, A., Martins, N., Selamoğlu, Z., Ahmad, Z., 2019. Allicin and health: a comprehensive review. Trends Food Sci. Technol. 86, 502–516. https://doi.org/10.1016/j.tifs.2019.03.003.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States-major pathogens. Emerg. Infect. Dis. 17 (1), 7–15.

Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., Škrlec, I., 2021. Candida albicans—The virulence factors and clinical manifestations of infection. J. Fungi 7 (2), 79. https://doi.org/10.3390/jof7020079.

Tedeschi, P., Nigro, M., Travagli, A., Catani, M., Cavazzini, A., Merighi, S., Gessi, S., 2022. Therapeutic potential of allicin and aged garlic extract in Alzheimer's disease. Int. J. Mol. Sci. 23 (13). https://doi.org/10.3390/ijms23136950.

Weiser, J.N., Ferreira, D.M., Paton, J.C., 2018. Streptococcus pneumoniae: transmission, colonization and invasion. Nat. Rev. Microbiol. 16 (6), 355–367. https://doi.org/10.1038/s41579-018-0001-8.

World Health Organization (WHO), 2018, February 7. *E. coli*. Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/e-coli.

Zhu, X.-Y., Zeng, Y.-R., 2020. Garlic extract in prosthesis-related infections: a literature review. J. Int. Med. Res. 48, (4). https://doi.org/10.1177/0300060520913778 030006052091377.