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Antimicrobial Activity of Garlic Extract on Organisms Isolated from Tomato Rot

Grace Michael Ikon[1], Victor Nelson Abasiubong[2] and Chijioke Patrick Amadi[2*]

[1]Department of Microbiology, Faculty of Natural and Applied Sciences, Obong University, Obong Ntak, Akwa Ibom State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, University of Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author VNA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GMI and CPA managed the analyses of the study. Author CPA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aqueous garlic extract is made up of antioxidant ingredient and has been responsible for boosting the immune system of the body; it is also used as a broad spectrum antibiotic, killing bacteria, fungus, and viruses. The microbial activities of spoilt and fresh tomato fruit was investigated using serial dilution method.

The antimicrobial activity of aqueous garlic extract against selected microorganisms isolated from spoilt tomato fruit was determined using diffusion method. The total heterotrophic bacteria count ranged from 4.0 X 10^5 cfu/g to 8.0 X 10^6 cfu/g and total coliform count ranged from 2.5 X10^5 cfu/g to 3.5 X 10^6 cfu/g and total fungal count ranged from 1.0 X 10^5 cfu/g to 2.6 X 10^6 cfu/g cfu/g. The bacteria isolated were *Salmonella entercolitis, Escherichia coli, Enterococcus faecium, Pseudomonas aeruginosa, Staphylococcus aureus, Acetobacter aceti, Xanthomonas campestris, Lactobacillus plantarum*, *Streptococcus pyogenes*, *Leuconostoc gasicomitatum*, while the fungi

**Corresponding author: E-mail: chijo42k@yahoo.com;*

isolates where *Mucor* spp, *Saccharomyces cerevisae*, *Penicillium notatum*, *Aspergillus flavus*. Concentrations of 10, 20, and 40 mg/ml of garlic aqueous extract were tested for their antimicrobial activity against some of the bacterial and fungal isolates from spoilt tomato. The result showed that aqueous concentrations of garlic extract between 10 to 40 mg/ml possess antimicrobial properties on the selected organism apart from *A. flavus,* where there is no zone of inhibition.
The presence of bacteria pathogens and fungi in tomatoes could pose a serious threat to health. Therefore treatment of tomato fruit with at least 40 mg/ml garlic aqueous extract could be used as a beneficial antimicrobial agent.

Keywords: Antimicrobial activity; garlic aqueous extract; organisms; tomato rot.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is a savory typical red edible fruit, that belongs to the family *Solanaceae*. They are frost sensitive, and for many cultivars, it takes about 3 to 4 months for the seedling to produce a mature tomato. It is an annual vegetative fruit crop produced in sub tropical countries including Nigeria. The average annual production of tomato is 13,000 tonnes while the worldwide yield is in the average of about 26,000 tonnes per year [1]. The crop originated from Mexico and was introduced to Europe in the 16th Century and later to West Africa by colonial settlers in early 1900 [2]. In Nigeria, tomato plays an important role in meeting domestic and nutritional food requirements, generation of income, foreign exchange earnings and creation of employment [3]. The crop is grown for both fresh domestic and export market but there is increasing demand for processed tomato products [4]. Tomato is highly regarded as a source of vitamin A and B [5]. It is about 20 to 25 mg ascorbic acid per 100 kg [6]. It is grown and eaten around the world. Tomato as fruit, are used in salads or cooked as a vegetable, processed into tomato paste (Ket Chup), sauce and puree. The nutritional value of tomato makes it a widely accepted vegetable by consumers. Fruits are rich in Calcium, Phosphorus, Magnesium, Copper, Niacin, Iron, Folate, Vitamin A, B6, Vitamin E, Vitamin B2, Vitamin C, and Carbohydrates [2]. Furthermore, the fruit has medicinal value as a gentle stimulant for kidneys, and washing off toxins that contaminate the body systems. It improves the status of dietary anti-oxidants (lycopene, ascorbic acid and phenols) in diet [7]. Tomato juice is known to be effective for intestinal and liver problem. The mineral content with 2232 to 2668 and iron range from 5.0 to 12.2 mg/kg [8]. In Nigeria, tomatoes are grown in large quantities and are seasonal; during the period of harvest the price of this commodity is low. It becomes highly exorbitant as soon as the harvest period is over due to its perishability. Tomatoes are attractive cash crop for small scale farmers, and its production provides potential source of employment to many rural and urban areas in Nigeria. The tomato fruits have been marketed freshly picked from the field and is the best selling fresh market vegetable crop [9]. Tomato production is constrained by factors such as poor pre-harvest practices, adoption of poor production techniques, rough handling and moisture condensation causing pathogen infestation. Despite the human need of tomato, damage as a result of post- harvest spoilage micro-organisms has been of serious concern. Microbial decay is one of the main factors that determine losses and compromises the quality of the produce. The extent of the losses especially through microbial decay has not been quantified in most areas and this has been quantified the results as short lived. According to Ogawa et al. [10], the most important fungi causing post-harvest diseases include; *Penicillium* spp, *Aspergillus* spp, *Alternaria* spp, *Botrytis cinerea*, *Monilinia lax*, and *Rhizopus stolonifer*. Attack by most organisms follows physical injury or physiological breakdown, and inadequate storage of the commodity. In a few cases, pathogens can infect apparently healthy tissues and become the primary cause of deterioration [11]. Due to the physiological form of fruits they deteriorate easily in transit and storage, especially under conditions of high temperature and humidity and as a result, heavy losses occur [12]. Bacterial organism such *Bacillus* spores are very heat-resistant and cause flat sour spoilage. Molds can grow on the surface of improperly processed tomato product and may eventually reduce the acidity to a point where botulism producing spore can grow and produce a deadly toxins. Other microorganisms that cause spoilage in tomato are saprophytic bacteria, *P. notatum*, *L. plantarum*, *A. niger*, *Penicillium itaculum*. The reduction of moisture content of food alone cannot sufficiently protect it from invasion of microorganism such as bacteria,

yeast and mold. It becomes necessary to introduce chemical preservation to aid in the control of these consistencies. Control of tomato fruit rot has been practiced by synthetic chemicals. Several types of synthetic chemicals have been used successfully to control the post-harvest decay of fruits and vegetables [13,14]. However there are three major concerns: (a) the increasing consumer concern over pesticide residues on foods [15]; (b) the predominance of fungicide resistant strains due to excessive use of fungicides [16,17]; (c) environmental pollution. Onuegbu [18], and Ramazani et al. [19], concluded that there is increased crop production by use of synthetic chemicals for management of plant pathogens, pests and weeds but with deterioration of environmental quality and human health. However, consumers demand less use of chemicals and still want food devoid of microbial growth, toxins as well as other quality deteriorating factors [20]. According to Bull et al. [21], Paster and Bullerman [22], synthetic fungicides such as thiabendazole, imazalil and sodium ortho-phenyl phonate have been used to control the post-harvest diseases, but their excessive use complemented with high costs, residues in plants, and development of resistance, has left a negative effect on human health and the environment. Efficient and effective control of seed borne fungi can be achieved by use of synthetic fungicides, but the same cannot be applied to fruits and vegetables.

Bio-control agents with extracts from plants such as lemon, citronella, clove, mint, thyme, and oregano oils have been used as alternatives to synthetic pesticides [23]. Adaskaveg et al. [13] and Serrano et al. [24] reported the use of garlic as a natural alternative to control *Penicillium digitatum.* Garlic is a perennial bulb-forming–plant that belongs to the genus *Allium* in the family *Lilliacease* along with leaks (*A. porrum*), Onions (*A. cepa*) and chives (*A. schoenoprasum*). For several centuries, garlic has been known to possess dietary and medical properties [25]. Several studies have proved that garlic has antimicrobial effects [26,27,28,29]. It inhibits the growth of both gram negative and gram positive bacteria the same as mold and yeast [30]. Garlic extract also reduced early blight disease on tomato [31]. According to Karapynar [32] garlic clove completely inhibited the mycelial growth of *A. flavus* and aflatoxin production. This study was aimed to determine the antimicrobial activities of garlic aqueous extract in different concentration against selected isolated microorganisms from spoilt tomato.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the Uyo, the capital of Akwa Ibom State Nigeria. Uyo metropolis has a population size of about 847, 500 and is seen by many as a fast growing city both economically and population wise. It is located in the rain forest belt with elevation of less than two feet above sea level. With its status as a developing capital city, it is surrounded by several sub-urban and rural communities. The maximum temperature experienced is between 26-28ºC, and annual rainfall of about 362.5 mm. The Latitude of Uyo, Nigeria is 5.038963, and longitude is 7.909470 with GPS coordinates of 5º 2' 0'' N and 7º 55' 0'' E and elevation of 65 m, 213 feet above sea level [33].

2.2 Collection of Samples

Samples of fresh tomato fruit and fresh garlic (*A. sativum*) were bought at Akpan Andem market.in Uyo metropolis; both vegetables were identified by a botanist in the department of botany, University of Uyo, where voucher number was assigned to the vegetative plants. The fresh tomato was allowed to spoil when kept unpreserved and immediately taken to microbiology laboratory for analysis using standard microbiological method. These samples were promptly processed within an hour of arrival to the Laboratory.

2.2.1 Isolation of microorganism from spoilt tomato fruit

The spoilt tomato fruits were blended. Thereafter, 1 g of tomato was weighed into 9 ml of sterile water and mixed properly. Ten (10) fold serial dilutions was carried out by drawing out 1 ml from the mixture using a sterile pipette and introducing in test tube containing 9 ml of sterile water which was finally diluted. One ml each from 10^{-4} and 10^{-5} was introduced into duplicates sterile petri dishes labeled for each of the following media, Nutrient agar, MacConkey agar and Sabouraud Dextrose Agar (SDA). Each medium cooled to about 45ºC and then was poured into petri dishes, swirled gently on the bench and allowed to set; Nutrient agar was used for bacterial count, MacConkey agar for total coliform bacteria count and Sabouraud Dextrose Agar for total fungal count. All bacteria plates were incubated. Coliform bacteria grew at temperature of 35±1°C. Fungi grew best at room temperature at room temperature for 5 to 7 days.

2.2.2 Purification and maintenance of stock cultures

With the aid of wire loop, a single distinct colony was picked from the 24 hours pure culture and incubated onto the surface of the agar slant, corked and incubated for 24 hours at 37ºC. The slant cultures transferred after incubation to a Laboratory refrigerator for preservation and maintenance [34].

2.2.3 Characterization and identification of isolates

Characterization and identification of the isolates were done as adopted by Cowman and Steel [35] Cruickshank et al. [36] and Cheesbrough [35].Gram stain, Catalase test, Coagulase test, Indole test, Citrate test, Oxidase test, Carbohydrate fermentation test.

2.2.4 Gram staining

Gram's stain on the bacterial isolates and lactophenol stain for fungal isolates was carried out.

2.2.5 Catalase test

Two drops of 3% freshly prepared hydrogen peroxide (H2O2) was placed on a grease free slide with aid of sterile glass rod, the 24 hours test organism was transferred onto the slide and observed immediately for gas bubbles, which indicates a positive reaction and non-production of gas bubble indicates negative result [34].

2.2.6 Coagulase test

A colony was emulsified in a drop of normal saline on grease free slide. A drop of human plasma using Pasteur's pipette was dropped and rotated for agglutination. It shows positive results for Coagulase test if there is visible clumping after 5 to10 seconds.

2.2.7 Indole test

Test tubes containing sterile peptone water was inoculated with the organism and incubated at 37ºC for 48 hours. One test tube that wasn't incubated with organism served as control. After incubation, 0.5 ml of KOVACS reagent was added, gently shaken and then allowed for 10 minutes, a positive result was characterized by formation of red color at the top layer.

2.2.8 Motility test

Semi-solid nutrient Agar in test tube was inoculated with the test organism inoculating wire loop by stabbing method. After inflaming the wire loop, the wire loop was then used to transfer the bacteria to the center of a cover slip as a small drop. The slide was inverted and observed under a light microscope using an immersion oil objective. Positive motility test was indicated by a diffuse zone of growth flaring from the line of inoculation. Negative motility test is indicated by a pinkish-red line that is confirmed to the stab line.

2.2.9 Citrate test

Simmon's citrated agar was prepared after which organism was inoculated for 24 hours at 37ºC. It was observed for color change from green to deep blue which is an indication of positive citrate utilization test.

2.2.10 Urease test

Using a sterile inoculating wire loop, the test organism was heavily inoculated over the surface of Christensen's medium on start and incubated at 37ºC for 24 hours. The culture was observed for change in colors from yellow to pink.

2.2.11 Oxidase test

A piece of filter paper was placed in a clean petri dish and 3 drops of freshly prepared Oxidase reagent was added. With the aid of sterile glass rod, the colony of test organism was removed and smeared on the filter paper. Positive result of Oxidase test was determined by observation of color change; blue to purple within 15 seconds.

2.2.12 Carbohydrate fermentation test

One gram of each test carbohydrate (Glucose, Lactose, Sucrose, Fructose and Manitol), was added to sterile based fermentation medium for each of the sugar mentioned above. To each medium, freshly prepared methyl red with sterile water was added before pipetting 10 ml of each medium into different test tubes, well labeled and Durham's tube inserted inverted in them, and then allowed to cool before inoculating the different isolates using a sterile inoculating loop, and then incubated at 37ºC for 24 hours. The test tubes were examined for the presence of gas and acid production. Acid production was indicated by changes in color of the medium from red to yellow while gas production was selected

by the presence of air space in the Durham's tube.

2.2.13 Garlic aqueous extract preparation

Fresh garlic bulb was obtained from local market. These were de-segmented and un-skinned. The segments were sterilized by washing with 0.2% sodium hydrogen peroxide for 2 minutes followed by 5 to 6 washing with distilled water. The sample was then ground in sterile pestle and mortar according to method of Kumar and Berwa [37]. After grinding, the following concentrations, 10, 20, and 40 mg/ml were prepared in sterile distilled water. Aqueous garlic extract was achieved using percolation extraction method.

2.2.14 Test for the antimicrobial effects of raw aqueous garlic extract: sensitivity for bacterial and fungal

A diffusion technique using Kirby Bauer method was applied in testing the isolates for their antimicrobial sensitivities. Nutrients broth was sterilized at 121ºC for 15 minutes and allowed to cool for 45ºC, then it was poured into a sterile MacConkey bottles. From the pure cultures, a distinct colony was picked and aseptically streaked into the nutrient broth using a sterile wire loop, and incubated at 37ºC for 6 hours. After 6 hours, culture was collected and aseptically inoculated evenly in each of the petri dish for the respective isolates. A cork-borer of 3 mm in diameter was used to borer 3-4 wells in each of the inoculated petri dishes, and the different concentration of garlic 10, 20, and 40 mg/ml were introduced into each of the well respectively, with one of the plates serving as a control that is without the concentration of garlic (0 mg/ml). The plates were incubated for 24 to 48 hours to observe for zones of inhibition. A Sabouraud Dextrose Agar (SDA) was sterilized at 121ºC for 15 minutes and allowed to cool for 45ºC, then about for 45ºC, then about 10 to 15 minutes was poured into sterile petri dish and allowed to set. The isolates (*Saccharomyce*, *Penicillum* spp) were aseptically picked using a sterile wire loop and inoculated into their respective plates. The isolates were evenly distributed, using the streaking method. Then a cork borer of 3 mm in diameter was used to bore 3 wells in each of the plates and the different concentrations of aqueous garlic extract, 10, 20, and 40 mg/ml of aqueous garlic extract were collected using micropipette. Then 2 to 3 drops of the different concentration from the micropipette was introduced into the respective wells. The plates were incubated at room temperature for 5 to 7 days to observe for clearance.

For the isolate (*Aspergillus*), 3 mm diameter cork borer inserted into the isolate was used to bore 3 to 4 wells repeatedly in aseptic condition, in a plate containing Sabouraud dextrose agar, then using a micropipette 2 to 3 drops of the different concentrations of garlic 10, 20 and 40 mg/ml were introduced into their respective wells. The plates were incubated at room temperature for 5 to 7 days in an upright position to observe for clearance. For isolates (*Saccharomyces* and *Penicillium* spp) in which there was zone of inhibition, the mycelia growth was measured away from the wells.

3. RESULTS

Table 1 shows the total heterotrophic, coliform and fungi counts of fresh and spoilt tomato fruit. Total coliform, hetetrophic, fungal count of fresh tomato served as the standard for food regulatory body in Nigeria.

4. DISCUSSION

The total heterotrophic bacteria count for both fresh and spoilt tomato ranged from fungal count ranged from 1.0×10^5–2.6×10^6. This is dissimilar to a previous study by Ogufure et al. [38]. In the previous study, the microbial count was high; this may be due to high microbial activity in spoilt tomato paste in can. Ten bacteria and four fungal species were found to be associated with tomato paste treated with different concentrations of garlic. The bacteria agents were *Streptococcus spp, Leuconostoc, Staphylococcus, Lactobacillus spp, Escherichia coli, Acetobacter, Xanthomonas and Pseudomonas spp.* The fungal isolates were *Aspergillus spp, Mucor, Saccharomyces and Penicillium spp.* Garlic concentrations of 10, 20, and 40 mg/ml were used in this research and the zone of inhibition obtained varies based on the concentrations. Mode of entry of microorganism into the tomato could be through scars, cracks and through soft point, also *Staphylococcus* being a normal flora of a human body can be transferred into the product of tomato during the preparation process. Treatment with *Allium sativum* at 10, 20 and 40 mg/ml aqueous concentrations inhibit the growth of microorganism from tomato paste within 24 hours. Enterotoxic *E. coli* strains and other pathogenic intestinal bacteria, which are diarrheagenic in humans and animals, have been reported to be easily inhibited by garlic [39].

Table 1. Total microbial count of fresh and spoilt tomato fruit

Sample	Total heterotrophic	Total coliform counts	Total fungal counts
Fresh Tomato	4.0×10^5 cfu/g	2.5×10^5 cfu/g	1.0×10^5 cfu/g
Spoilt Tomato	8.0×10^6 cfu/g	3.5×10^6 cfu/g	2.6×10^6 cfu/g

Table 2. Biochemical and morphological characterization of bacteria

S/N	Microscopic observation	Gram stain	Spore coagulase	Oxidase	Indole	Motility	Methyl red	Vogue proskaue	Citrate	Urease	Motility	Glucose	Sucrose	Lactose	Fructose	Manitol	Probable organism
1	Rod	+	-	-	-	-	-	-	-	-	-	AG	AG	AG	AG	AG	*Leuconostoc gasicomitatum (Spoilt)*
2	Cocci	+	-	+	-	+	+	+	-	+	-	A	A	A	A	A	*Staphyloccus aureus (Spoilt)*
3	Cocci	+	-	-	+	-	-	-	-	+	-	A	A	A	A	A	*Streptococcus pyogenes(Spoilt)*
4	Cocci	+	-	-	-	-	-	+	+	-	+	A	A	A	A	A	*Enterococcus faecium(Spoilt)*
5	Rod	+	-	-	-	+	+	-	-	-	-	AG	-	AG	AG	AG	*Lactobacillus plantarum(Spoilt)*
6	Rod	-	-	-	-	+	+	-	+	-	+	AG	AG	AG	AG	AG	*Salmonella(Spoilt)*
7	Rod	-	-	-	+	+	+	-	-	-	-	AG	A	A	A	A	*Escherichia coli (Spoilt)*
8	Rod	+	-	-	-	-	-	+	-	-	+	AG	-	-	-	-	*Xanthomonas campestris(Spoilt)*
9	Rod	+	-	+	-	+	+	+	-	-	+	AG	AG	AG	AG	AG	*Acetobacter aceti (Spolt)*
10	Rod	+	-	-	+	-	-	-	+	-	+	AG	AG	AG	AG	AG	*Pseudomonas aeruginosa(Spoilt)*

A = Acid; Ag= Acid and gas; += Positive; -= Negative

Table 3. Morphological characterization fungi isolates

Color	Morphology	Nature of hyphae	Spore shapes	Reproductive structure	Probable organism
White	White colony	Long erection septate	Smooth and regular and *sporogiosphore*	Multi nucleate globose vesicle with radiating sterigria	*Mucor* spp
Creamy white	Smooth colony	Non-septate hyphae	Ellipsolidial	Multinucleated globose vesicle with *conida*	*Saccharomyces*
Black	Presence of aerial hyphae	Non-septate	Oval	Upright *conidiophores* that are simple and terminate in a globose	*Aspergillus* spp
Greenish blue	Brush like tip of shot *sporangiophore*	Septate	Oval	Long *conidiosphor* es with chains of spores	*Penicillium* spp

Table 4. Antimicrobial effect of concentrations of aqueous garlic against selected organism

Conc_ of acqueous garlic extract (mg/ml)	*E. coli* (mm)	*Pseudomonas* spp (mm)	*Xanthomonas* spp (mm)	*Streptococcus* spp (mm)	*Lactobacillus* spp (mm)	*Penicillium* spp (mm)	*Saccharomyces* spp (mm)	*Aspergillus* spp (mm)
(Control) 0 mg/ml	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
10	3	14	11	15	9	10	9	No inhibition
20	16	17	14	19	14	18	14	No inhibition
40	20	22	19	22	18	21	19	No inhibition

The absence of resistance to garlic enhances its ability to effectively act against even highly resistant bacteria strains, such *Enterococcus* and *Pseudomonas aeruginosa,* it therefore appears that antibiotics that affect DNA and RNA synthesis could form an effective combination [40]. The minimum inhibitory concentrations (MIC); 40 mg/ml of aqueous garlic extract on *Staphylococcus aureus* was determined to be greater than 7.5 mg/ml which is similar with the study of Shokradeh and Ebadi [41]. This may be due to the biological conditions. All clinical isolates of *S.aureus* were tested on garlic extract which was autoclaved at 121ºC for 15 minutes.

Table 5. Garlic aqueous extract Tube Dilution Test (determination of minimum inhibitory concentration)

Conc_ of acqueous garlic extract (mg/ml)	Garlic aqueous extract
10	No inhibition
20	No inhibition
40	-

N.B = -Growth Observed (not turbid)
Minimum Inhibitory Concentration. Garlic acqeous extraction = 40 mg/ml

There was no antibacterial effect in contrast to the study of Shokradeh and Ebadi [41]. The antimicrobial effect of crude preparation of garlic at room temperature (Fresh garlic) and refrigerated at -10ºC has the same antibacterial effect, however; the fresh garlic shows greater effectiveness. On considerations of the problems, the study focused on the garlic antibacterial 294 activity on *S. aureus* and revealed the dilute solution of garlic completely inhibit the growth of *S. aureus* at the concentrations of more than 7.50 mg/ml. This could be due to the actions of biological active ingredient of *allicin* on *S. aureus* inhibit synthesis to prevent chromosomal replication. Although DNA and protein synthesis are also partially inhibited, suggesting that RNA is the primary target of allicin action [42].

Garlic has been in use since ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease [43,44, 45], and regular use of garlic may help to prevent cancer, treat malaria and to raise immunity. Garlic has also proposed to treat asthma, Candidiasis, colds, diabetes and antibacterial effect against food borne pathogens like *Salmonella, Shigella* and *S. aureus* [46].

The antimicrobial activity of aqueous garlic has been attributed to the presence of thiosulfinates (e.g, *allicin*) whose removal completely renders garlic ineffective against microorganism [47].

5. CONCLUSION

Bacterial and fungal isolates of spoilt tomato was observed through microscopic analysis. Morphological characterization of test organisms was conducted; positive and negative results were obtained during this study. Minimum inhibitory concentration of garlic aqueous extract was determined. Result of the finding in this study, showed that garlic aqueous extract concentration at 10, 20 and 40 mg/ml reduced microbial load in spoilt tomato paste for a period of 24 hours for the bacteria isolates and 5-7 days for fungal isolates. From this research findings, garlic has been able to exhibit antimicrobial activity against organism isolated from spoilt tomato paste. Due to the medicinal potency of garlic, the aqueous extract of garlic can be recommended to pharmaceutical industries, for manufacturing new antibiotics drugs that can be susceptible to *S. aureus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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