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Susceptibility of Some Clinically Resistant Bacterial Isolates to Thyme Essential Oil, Chitosan and *Lactobacillus reuteri*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Numerous avenues are currently being investigated to curtail multidrug resistance. The antibacterial activity of naturally derived agents, including thyme essential oil, chitosan and a probiotic *Lactobacillus* strain was screened against clinically resistant isolates of *Staphylococcus aureus, Escherichia coli* and *Klebsiella.* Thyme essential oil was hydro-distilled from wild *Thymus capitatus* and its chemical composition was profiled by Gas Chromatography-Mass Spectroscopy. Chitosan was prepared from shrimp shells and characterised by infrared spectroscopy. The antibiotic sensitivity pattern of the clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* were investigated, and their susceptibility to the prepared natural agents was assessed by agar well diffusion technique. Both Gram-negative isolates exhibited similarly high resistance rates

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to piperacillin, co-amoxiclav, cephalothin, ceftazidime and imipenem. Meanwhile, 83% of *S. aureus* isolates were of the MRSA phenotype. The tested isolates displayed varying degrees of susceptibilities to the tested natural agents where thyme essential oil exhibited the highest inhibitory effect followed by chitosan and the culture supernatant of *L. reuteri* respectively. Albeit the high resistance displayed by both *E. coli* and *Klebsiella* isolates, they were the most susceptible to thyme essential oil. Contrariwise, *L. reuteri* exhibited the highest inhibitory effect against *S. aureus* isolates. Intriguingly, there was a general tendency for higher effectiveness of the natural products tested against the most resistant isolates implying that these natural products may have a resistance modifying potential. The presently investigated natural agents hold a promising potential against clinically significant multidrug resistant bacteria.

Keywords: Antibacterial; resistant clinical isolates; thyme essential oil; chitosan; Lactobacillus reuteri.

1. INTRODUCTION

Globally, there is an escalating incidence of infections caused by resistant Gram-positive and Gram-negative pathogens in the nosocomial environment and the community. This growing number of antibiotic-resistant pathogens places a significant burden on healthcare systems and imposes global economic burden [1]. The infectious diseases society of America has highlighted a fraction of antibiotic-resistant bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*) - acronically dubbed the "ESKAPE" pathogens- which are capable of escaping the biocidal activity of antibiotics and mutually representing new paradigms in pathogenesis, transmission and resistance [2]. Although a lot of emphasis is placed on the major health threats of MRSA, an even greater threat looms for the prevalence of MDR Gram-negative bacteria. Mortality rates from these increasingly resistant pathogens have surpassed those caused by H.I.V. and T.B. combined [3].

Antibiotics have played a pivotal role in achieving major advances in medicine. They have not only saved patients' lives, extended the expected outcomes of bacterial infections but have also prevented infections in immune-compromised patients or those who have undergone surgeries [4]. In developing countries, where sanitation is still poor, antibiotics decrease the morbidity and mortality caused by food-borne and other poverty related infections [5]. The escalating rise of antimicrobial resistance revokes the health benefits that have been achieved by antibiotics and is unmet with a lack of new antimicrobials production [6].

The problem of antimicrobial resistance is a demanding issue that strains health care resources. Recently, antimicrobial research is geared toward natural agents. The plethora of natural agents derived from plants, animals and microorganisms is vast [7]. Essential oils derived from plants represent a notable source for antimicrobials and studies reported that their antimicrobial activity is not attributable to a unique mechanism but is instead due to a cascade of reactions involving the entire bacterial cell [8]. Antimicrobials from microorganisms and animals have not received much attention as plants. The antibacterial activity of probiotics is an emergent topic and their potential to be used as an adjunct in the control of antibiotic resistance is particularly appealing [9]. Chitosan, the most abundant biopolymer, presents an inherent antimicrobial character against the growth of pathogenic microorganisms in a range of foods. Although the exact mechanism by which chitosan exerts its antimicrobial action has not been fully elucidated, it has been postulated to be dependent on its molecular weight and deacetylation degree [10,11]. In view of the declare of antimicrobial resistance as one of the greatest threats to human health and the dearth for new antimicrobials, the present study aimed at screening the antimicrobial potentiality of thyme essential oil, chitosan and a probiotic bacterial strain towards clinically resistant isolates of *Staphylococcus aureus, Escherichia coli* and *Klebsiella*.

2. MATERIALS AND METHODS

2.1 Plant Collection, Essential Oil Extraction and Compositional Analysis

The aerial shoots of wild thyme were collected, during September 2014, from rocky ridge habitats in Burg El-Arab area, Alexandria, Egypt and were taxonomically identified at the Botany

Department, Faculty of Science, Alexandria University. Thyme essential oil was hydro-distillated from the dried aerial parts using Clevenger type apparatus, characterized using GC-MS (Thermo-scientific ISQ, Quadropole MS/Trace GC Ultra). The carrier gas was helium at a flow rate of 1 ml/min and the oven temperature was kept at 60°C for 3 min then programmed to 220°C at a rate of 5°C/min, the injector temperature was set at 250°C and mass spectra were taken at an ionisation voltage of 70 ev. Qualitative identification of the oil constituents was carried out on the basis of their retention indices and matching their mass spectral fragmentation patterns with NIST library database [12]. Quantitative analysis of the oil components, expressed as relative percentage of peak area, was estimated using peak area normalisation measurements.

2.2 Preparation and Characterisation of Chitosan

Chitosan was prepared using crustacean shells of shrimp which were scrapped free of loose tissue, washed with distilled water and dried in air [13]. The ground shells were demineralised by agitating with 10% HCL, deproteinized with 10% NaOH and chitin was deacetylated by heating at 100-120°C using 50% NaOH at a ratio of 1:20 w/v for 5 hr. One percent of the ground chitosan was dissolved in 1% acetic acid solution, agitated overnight and autoclaved at 121°C for 15 min. All chemicals used in this study were of analytical grade.

Functional groups of chitosan were characterised using Fourier Transform Infrared spectroscopy (Perkin Elmer US/Spectrum BX spectrometer) by KBr pellet technique. The degree of deacetylation of the prepared chitosan was calculated according to the following formula [14]:

$$DD\% = 100 - \frac{(A_{1660\,cm^{-1}})/(A_{3450\,cm^{-1}})}{1.33} * 100$$

Where A_{1660} and A_{3450} are the absorbance at 1660 cm^{-1} and 3450 cm^{-1}, respectively.

2.3 Bacterial Isolates

The tested isolates were recovered from clinical specimens of blood, bronchoalveolar lavage (BAL), urine and pus specimens, nasal and throat swabs obtained from a local governmental hospital. Samples were surface plated onto blood and MacConkey agar (Oxoid), incubated aerobically at 37°C for 24 hr. The isolates were identified by their colonial morphology, Gram-staining and their biochemical reactions according to CLSI guidelines [15]. For identification of *S. aureus,* the isolates were streaked onto Mannitol salt agar and human plasma was used for coagulase confirmation.

2.4 Antibiogram of the Clinical Isolates

The antibiotic susceptibility of 12 isolates from each species was assessed using Kirby Bauer disc diffusion assay [16]. The isolates were cultured overnight in nutrient broth (Oxoid). The bacterial suspension was adjusted to 0.5 McFarland turbidity standard using sterile saline, swabbed onto Mueller Hinton agar plates (MHA, Oxoid). The sensitivity of *S. aureus* isolates were tested to Cefoxitin (30 µg), Erythromycin (15 µg), Sulphamethoxazole/Trimethoprim (25 µg), Linezolid (30 µg), Vancomycin (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Doxycycline (30 µg) and Tobramycin (10 µg), whereas Piperacillin (30 µg), Amoxicillin/ Clavulanic acid (30 µg), Cephalothin (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Aztreonam (30 µg), Meropenem (10 µg), Imipenem (10 µg), Levofloxacin (5 µg) and Gentamicin (30 µg) discs (Oxoid) were used for sensitivity testing of *E. coli* and *Klebsiella* isolates. The inoculated plates were incubated at 37°C for 24 hr. The inhibition zone diameters around the discs were measured and interpreted according to the CLSI guidelines, 2015 [17]. Reference strains of *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Staphylococcus aureus* ATCC 25923 were used.

2.5 Antibacterial Activity Testing

The sensitivity of the isolates to thyme essential oil, chitosan and the probiotic bacterial strain (*Lactobacillus reuteri* ATCC 53608) was screened using agar well diffusion technique [18]. The respective bacterial suspension, adjusted to 0.5 McFarland, was swabbed over MHA plates and wells of 6 mm diameter were punched in the agar plates using a cork borer. Fifteen µl of thyme essential oil was added to the wells of MHA plates containing 0.5% Tween 20 (Sigma) to enhance oil diffusion. Similarly, 15µl of 1% chitosan in 1% acetic acid solution or 15µl of probiotic culture supernatant were instilled into the wells of MHA plates without Tween. Plates were left for 30 min. and then were incubated at

37°C for 24 hr. The diameter of the growth inhibition zone was measured in mm and the mean value of triplicate plates was calculated.

3. RESULTS

3.1 Chromatogram of *Thymus capitatus* Essential Oil

Gas chromatography-mass spectroscopic analysis of the extracted *Thymus capitatus* essential oil revealed that thymol, carvacrol and *p*-cymene were the major components constituting 43.34%, 13.17% and 11.32% respectively. Other components constituting the oil included the terpene hydrocarbons; *β*-pinene, *γ*-terpinene and *β*- caryophyllene comprising 1.53%, 1.62% and 2.25% correspondingly. Moreover, the alcoholic terpenes; borneol and linalool displayed a relative abundance of 3.2% and 1.7% as shown in Table 1 and Fig. 1.

3.2 FTIR Spectrum of Shrimp Chitosan

The structural properties of chitosan prepared from crustaceans shells of shrimp were characterised using FTIR spectroscopy. As shown in Fig. 2, various absorption bands within 4000 to 400 cm^{-1} were recorded in the FTIR spectra. The first characteristic peak at 3484 cm^{-1} characterises N-H in NH_2 association in primary amines or is ascribed to O-H in OH association in pyranose ring. The other characteristic peak was that at 1660 cm^{-1}, that is attributed to the C=O stretching in $NHCOCH_3$ group. The presence of absorption bands at 2962 cm^{-1} characterises the C-H stretch in CH_2OH group, whereas the peak at 1416 cm^{-1} characterises the C-H bending in the same group. Moreover, the absorption band at 1142 cm^{-1} is characteristic for the glycosidic linkage in the prepared polymer. Using the absorbance values at wavelengths 1660 cm^{-1} and 3450 cm^{-1}, the deacetylation degree (DD) for shrimp chitosan was 73%.

Table 1. Major components of thyme essential oil as identified by Gas Chromatography-Mass Spectroscopy

Component	Retention time (min.)	Relative abundance (%)	Molecular formula
γ-terpinene	11.25	1.62%	C10H16
β-pinene	13.90	1.53%	C10H16
p-cymene	14.44	**11.32%**	C10H14
Borneol	19.83	3.2%	C10H18O
Linalool	24.56	1.7%	C10H18O
Carvacrol	24.9	**13.17%**	C10H14O
Thymol	25.85	**43.34%**	C10H14O
β-caryophyllene	28.53	2.25%	C15H24

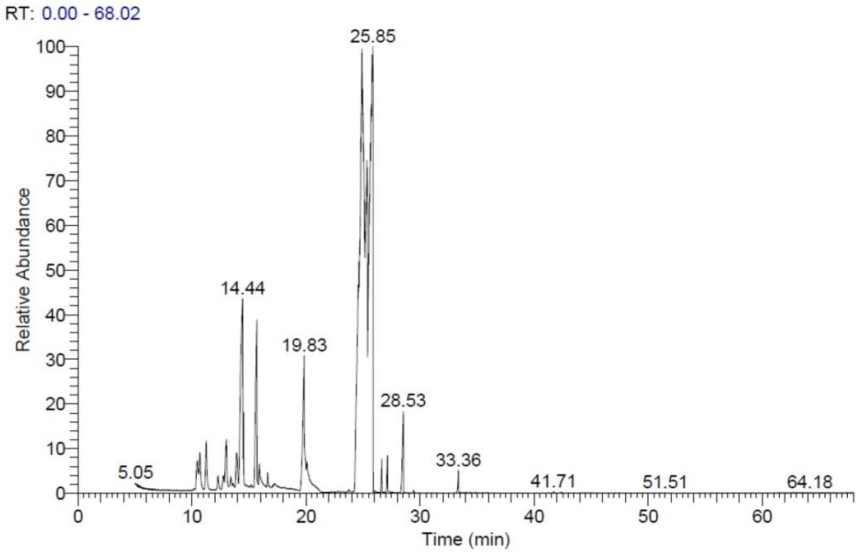

Fig. 1. Chromatogram of *Thymus capitatus* essential oil

3.3 Antibiogram of the Clinical Isolates

As demonstrated in Fig. 3, 83% of the investigated *S. aureus* isolates were methicillin resistant. None of the isolates was resistant to linezolid, vancomycin or tobramycin. The highest resistance was exhibited to erythromycin and tobramycin comprising 75%. Half the isolates were resistant to amikacin and 25% exhibited resistance to co-trimoxazole (25%).

The overall resistance of the tested *E. coli* isolates exceeded 90% for penicillins and cephalosporins (piperacillin, co-amoxiclav, meropenem, aztreonam, cephalothin, cefotaxime, ceftriaxone and ceftazidime). Meanwhile, resistance rates decreased in the order of imipenem, levofloxacin and gentamicin comprising 41.7%, 33.3% and 16.7% respectively. Comparative to *E. coli, Klebsiella* isolates demonstrated higher resistance rates to levofloxacin and gentamicin comprising 83% and 75% versus 33.3% and 16.7%. On the contrary, resistance to aztreonam was lower than that exhibited by *E. coli* (75% versus 91.7%). Meanwhile, both isolates displayed similar resistance rates to piperacillin, co-amoxiclav, cephalothin, ceftazidime and imipenem (Fig. 4).

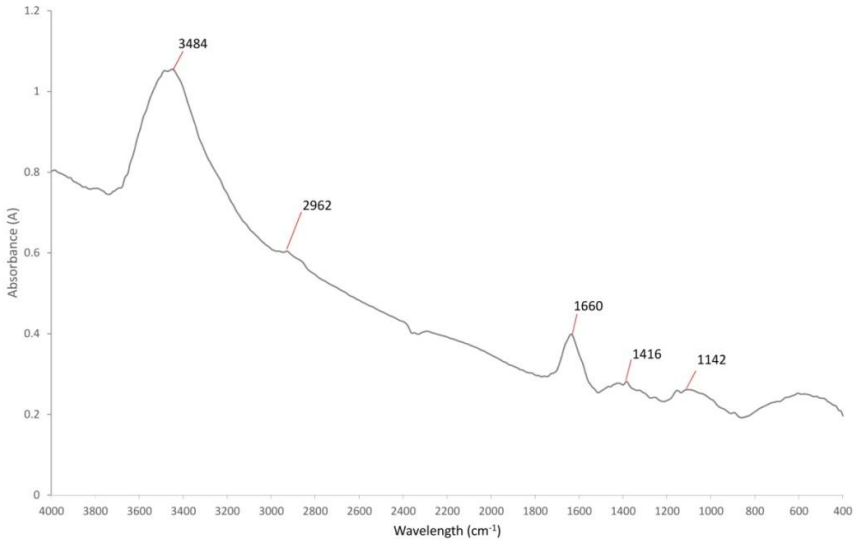

Fig. 2. FTIR spectrum of shrimp chitosan

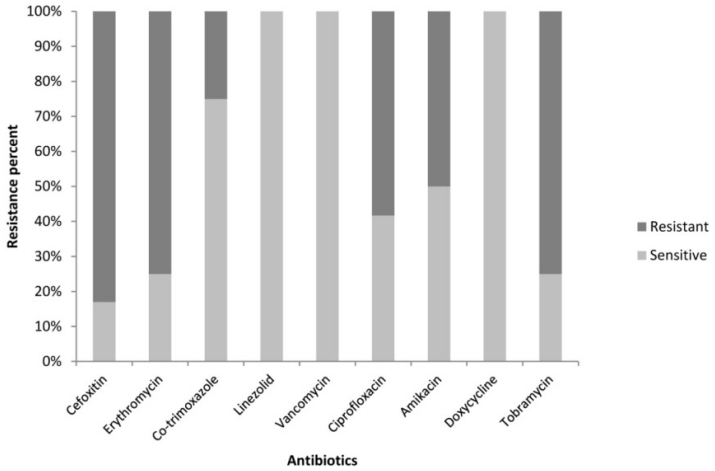

Fig. 3. Antibiotics resistance pattern of *Staphylococcus aureus* isolates

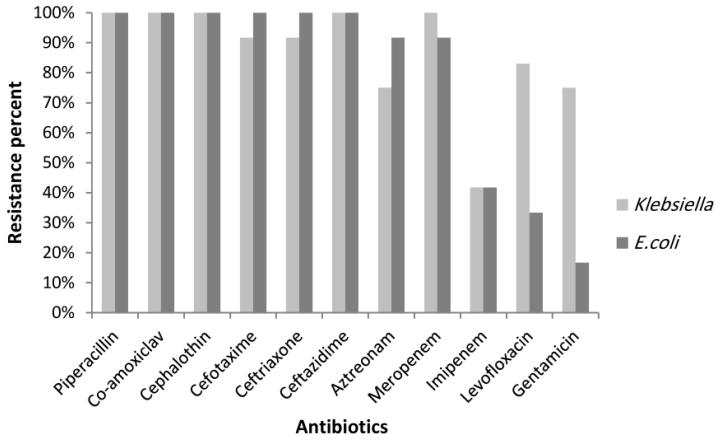

Fig. 4. Antibiotics resistance pattern of *Klebsiella* and *Escherichia coli* isolates

3.4 Susceptibility of the Clinical Isolates to *Thymus capitatus* Essential Oil, Chitosan and *Lactobacillus reuteri*

The investigated bacterial strains displayed varying degrees of susceptibility to the tested natural products. Thyme essential oil exhibited the highest activity towards *E. coli* isolates followed by *Klebsiella* and *S. aureus* with a mean inhibition zone of 25.8 mm, 18.3 mm and 15.3 mm respectively. Chitosan displayed higher activity against *E. coli* with a mean inhibition zone of 14.8 mm versus 12.3 mm for *Klebsiella.* However, *S. aureus* isolates were the least susceptible with a mean inhibition zone of 11.7 mm. On the contrary to thyme oil and chitosan, *Lactobacillus reuteri* exhibited the highest activity against BAL staphylococcal isolates and the least effect against *Klebsiella* pus isolates. The presently investigated clinical *E. coli* isolates displayed nearly similar sensitivity to *L. reuteri* except for urine isolates which displayed an inhibition zone range of 8 to 14 mm irrespective of their similar antibiotic sensitivity patterns (Table 2).

4. DISCUSSION

The resistance of bacteria to antibiotics has escalated radically, over the past years, to incidences where some Gram-negative bacilli were resistant virtually to all known antibiotics [19]. The overall resistance rates of the currently investigated *E. coli* isolates are in agreement with the general global trend of resistance of *E. coli* to antibiotics [20,21,22,23]. In a one year retrospective survey of antimicrobial resistance in five hospitals in Cairo, *E. coli* isolates were reported to resist most antimicrobials with the least resistance cited for imipenem, ciprofloxacin and gentamicin [24]. This is in agreement with the present results since the isolates displayed the least resistance rates to levofloxacin and gentamicin (Fig. 3). *Klebsiella* resistance pattern, in the present study, surpasses those reported in other studies conducted in Nigeria, Iran and India [25,26,27]. This variability is probably attributed to differences in antibiotic selection pressure, local antibiotics and prescribing habits which differ between countries.

Globally, defining antimicrobial resistance in *S. aureus* has been agreed upon as instantly classifying an isolate that is resistant to methicillin as an MDR [28]. It is reported that MRSA isolates despite being resistant to all categories of betalactam antibiotics, they also display resistance to other classes of antibiotics comprising aminoglycosides, tetracyclines, folate pathway inhibitors, fluoroquinolones and macrolides with variable degrees [29]. In the current study, multiple antibiotic resistance was also implicated (Fig. 4). Following cefoxitin, erythromycin and tobramycin were the antibiotics to which the isolates showed the highest resistance. Moreover, approximately 60% of the isolates were resistant to the fluoroquinolone "ciprofloxacin" and 50% were resistant to the aminoglycoside "amikacin". On the contrary, the studied isolates were sensitive to vancomycin and linezolid, which is in agreement with the results of several investigators reporting the sensitivity of *S. aureus* to relatively newer antimicrobials [30].

Table 2. Antimicrobial activity of *Thymus capitatus* essential oil, chitosan and *L. reuteri* against clinical isolates of *S. aureus*, *E. coli* and *Klebsiella spp*.

***Staphylococcus aureus* (S)**				***Escherichia coli* (E)**				***Klebsiella* (K)**			
Source (R)*	**Thyme EO**	**Chitosan**	***L. reuteri***	**Source (R)***	**Thyme EO**	**Chitosan**	***L. reuteri***	**Source (R)***	**Thyme EO**	**Chitosan**	***L. reuteri***
ThS1 (R5)	14±0.1	12±0.4	9±0.1	BALE1 (R10)	24±0.3	12±0.1	10±0.5	BALK1 (R11)	18±0.9	11±0.9	11±0.2
ThS2 (R5)	15±0.3	14±0.5	14±0.3	BALE2 (R8)	16±0.4	13±0.2	11±0.1	BALK2 (R11)	20±0.2	13±0.1	8±0.1
ThS3 (R0)	12±0.2	11±0.7	10±0.9	BALE3 (R9)	25±0.3	12±0.3	9±0.7	BALK3 (R11)	18±0.1	11±0.2	7±0.5
BALS1 (R5)	18±0.1	12±0.2	19±0.2	UrE1 (R8)	29±0.5	15±0.9	14±0.2	UrK1 (R9)	19±0.5	12±0.4	10±0.4
BALS2 (R3)	12±0.2	14±0.2	17±0.4	UrE2 (R8)	26±0.4	12±0.7	8±0.4	UrK2 (R8)	16±0.6	11±0.2	10±0.5
BALS3 (R1)	13±0.4	11±0.1	18±0.3	UrE3 (R8)	37±0.5	17±0.3	10±0.1	UrK3 (R10)	21±0.1	13±0.1	7±0.7
BS1 (R3)	17±0.1	12±0.3	15±0.5	BE1 (R9)	27±0.4	15±0.6	11±0.1	BK1 (R10)	18±0.2	11±0.7	14±0.5
BS2 (R4)	17±0.3	10±0.7	20±0.6	BE2 (R9)	26±0.2	14±0.5	12±0.1	BK2 (R9)	15±0.4	17±0.2	10±0.1
BS3 (R2)	16±0.5	12±0.2	10±0.1	BE3 (R11)	20±0.1	15±0.6	11±0.3	BK3 (R11)	20±0.5	13±0.3	13±0.4
NaS1 (R4)	16±0.7	9±0.8	13±0.2	PE1 (R7)	27±0.2	11±0.2	10±0.4	PK1 (R5)	14±0.2	11±0.4	9±0.2
NaS2 (R6)	19±0.8	14±0.4	12±0.3	PE2 (R10)	30±0.4	14±0.1	11±0.2	PK2 (R10)	20±0.9	13±0.5	7±0.4
NaS3 (R6)	14±0.9	13±0.3	18±0.2	PE3 (R8)	22±0.2	15±0.6	10±0.5	PK3 (R10)	21±0.1	12±0.3	7±0.2
IZ** range	12-19	9-14	9-20	IZ** range	16-37	11-17	8-14	IZ** range	14-21	11-17	7-14
Mean IZ	15.25	11.72	14.58	Mean IZ	25.75	14.75	10.5	Mean IZ	18.33	12.33	9.42
ATCC 25923	28±0.3	10±0.5	11±0.2	ATCC 25922	21±0.3	12±0.3	14±0.5	ATCC 13883	35±0.4	14±0.2	14±0.6

**Source expressed in terms of the clinical specimen from which the organism (S, E or K) was recovered. Th: throat swab; Na: nasal swab, B: Blood, Ur: urine, P: pus and BAL broncheo-alveolar lavage isolates of the respective clinical specimens.*

(R) drug resistance pattern expressed in terms of the no. of antibiotics to which the isolate was resistant amongst all the tested antibiotics; R0=sensitive to all tested antibiotics; R1 to R11- resistant to one, two, three etc. antibiotics respectively.

***IZ is the inhibition zone diameter in mm expressed as mean values ± standard error of means of three experiments*

The influence of essential oils on Gram-positive and Gram-negative pathogens is controversial. Some investigators proposed that both Gram-positive and Gram-negative bacteria are indifferently susceptible to plants' essential oils [31]. Others reported that Gram-negative bacteria are more sensitive to the antibacterial action of essential oils [32,33], whereas the majority of investigators reported that Gram-positive pathogens are more susceptible to essential oils [8,34,35,36,37]. In line with the studies reporting that Gram-positive pathogens are more resistant to essential oils, the present results conveyed that both *E. coli* and *Klebsiella* isolates were more sensitive to thyme essential oil than *S. aureus* (Table 2). As demonstrated from the compositional analysis of the presently extracted essential oil, thyme oil is dominated by thymol (43%) which is a phenolic terpene that is reported to exhibit an antimicrobial effect via multiple pathways affecting a variety of cellular functions [38]. Accordingly, the displayed inhibitory effect against the tested isolates is probably attributed to thymol in addition to synergy of other components constituting the oil (Table 1, Fig. 1). Intriguingly, in the present study, thyme oil exhibited variable activities in relation to the isolates' resistance pattern. A maximum inhibitory effect was exhibited against a nasal staphylococcal isolate that is resistant to 6 of the 11 tested antibiotics; on the contrary a throat isolate that is sensitive to all the tested antibiotics was the least sensitive to the oil. The same was true in case of *E.* coli, where the least effect was exhibited towards the most resistant isolates. Simultaneously, the most resistant *Klebsiella* isolates displayed the highest susceptibility to the oil (Table 2). In this context, limited research has been done for the exploration of the capability of plant extracts in modulating bacterial resistance. Some phytochemicals have been reported to possess resistance modifying activity *in vitro* and some have been reported to reverse betalactam resistance in MRSA [39]. In the current study, the general tendency for higher effectiveness of *Thymus capitatus* essential oil against the highly resistant isolates implies that thyme oil may have a resistance modifying potential.

Chitosan was more effective against both *Klebsiella spp*. and *E. coli* than *S. aureus* (Table 2). In literature, the effectiveness of chitosan on Gram-positive or Gram-negative bacteria is however somewhat controversial. Some studies have reported that chitosan generally showed stronger effects against many Gram-positive bacteria including *S. aureus* [40,41,42]. On the contrary, in several *in vitro* studies, Gram-negative bacteria appeared to be very sensitive to chitosan compared to Gram-positives [43,44,45]. This difference in effectiveness has been proposed to be affected by the charge density on the cell surface in addition to the degree of deacetylation which has been postulated as an important determinant for chitosan's antimicrobial effectiveness [46]. Accordingly, in the present study, it can be conceived that shrimp chitosan (DD 73%) displayed an effect that is relatively higher than that reported in other studies [47,48], which can be counted for in terms of the difference in their deacetylation degrees.

The antagonistic activity of lactic acid bacteria against pathogenic strains has been documented and was reported to be exclusively active against Gram-positive bacteria [49,50]. In the current study, the culture supernatant of *Lactobacillus reuteri* showed a higher inhibitory effect towards *S. aureus* isolates than *E. coli* or *Klebsiella* (Table 2). The exhibited antagonistic effect may be counted for in terms of the bacterial growth inhibitory principles produced by *Lactobacilli.* These antimicrobial substances are not only restricted to organic acids but also include bacteriocins which were proposed to also inhibit Gram-negative bacteria when their cell surface structure is injured [51].

5. CONCLUSION

The presently investigated natural products derived from botanical, zoological origins and probiotic bacteria displayed various activities towards the tested resistant isolates of *Staphylococcus aureus, Escherichia coli* and *Klebsiella spp.* Mostly, isolates that displayed multiple antibiotic resistance were the most susceptible to the tested natural agents. Accordingly, they may have potential against resistant pathogens, nevertheless detailed studies of their mode of action and resistance modifying potential are warranted.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74:417-433.

2. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther. 2013;11:297-308.
3. Lee CR, Cho IH, Jeong BC, Lee SH. Strategies to minimize antibiotic resistance. Int J Env Res Public Health. 2013;10:4274–4305.
4. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol. 2010;1:134.
5. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Curr Opin Pharmacol. 2014;18:56–60.
6. Piddock LJ. The crisis of no new antibiotics—what is the way forward? Lancet Infect Dis. 2012;12:249–253.
7. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can overcome microbial resistance. Virulence. 2013;4: 185–191.
8. Nazzaro F, Fratianni F, De-Martino L, Coppola R, De-Feo V. Effect of essential oils on pathogenic bacteria. Pharm. 2013; 6:1451-1474.
9. Rijkers GT, De-Vos WM, Brummer RJ, Morelli L, Corthier G, Marteau P. Health benefits and health claims of probiotics: Bridging science and marketing. Br J Nutr. 2011;106:1291–1296.
10. Je JY, Kim SK. Chitosan killed bacteria by disrupting the outer and inner membrane. J Agric Food Chem. 2006;54: 6629-6633.
11. Limam Z, Selmi S, Sadok S, El-Abd A. Extraction and characterization of chitin and chitosan from crustacean by-products: Biological and physicochemical properties. Afr J Biotechnol. 2011;10:640-647.
12. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th Ed. Illinois: Allured Publishing Corp; 2007.
13. Roberts GAF. Preparation of chitin and chitosan. United Kingdom: Macmillan London Press; 1992.
14. Baxter A, Dillon M, Taylor KD, Roberts GA. Improved method for IR determination of the degree of N-acetylation of chitosan. Int J Biol Macromol. 1992;14:166–169.
15. CLSI, Clinical and Laboratory Standard Institute. Abbreviated identification of bacteria and yeast; Approved Guideline-Second edition. CLSI document M35-A2; 2008.
16. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45:493–496.
17. CLSI, Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplements. Approved Standard. CLSI document M100-S25; 2015.
18. Barry AL. Procedure for testing antimicrobial agents in agar media. In: Lorin V editors. Antibiotics in laboratory medicine. Baltimore: Williams Wilkins; 1980.
19. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. PT 2015; 40:277-283.
20. Pfaller MA, Jones RN, Marshall SA, Coffman SL, Hollis RJ, Edmond M. Inducible Amp-C-*β*-lactamase producing Gram-negative bacilli from bloodstream infections: Frequency, antimicrobial susceptibility and molecular epidemiology in a national surveillance program (SCOPE). Diagn Microbiol Infect Dis. 1997; 28:211–219.
21. Oplustill CP, Nunes R, Mendes C. Multicenter evaluation of resistance of *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella spp*. and *Shigella spp*. isolated from clinical specimens in Brazil: Resistant surveillance program. Braz J Infect Dis. 2001;5:8-12.
22. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended spectrum *β*-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. Clin Infect Dis. 2001;32:94–103.
23. Kibret M, Aberas B. Antimicrobial susceptibility patterns of *E. coli* from clinical sources in Northeast Ethiopia. African Health Sci. 2011;11:40-45.
24. El-Kholy A, Bassem H, Hall GS, Procop GW, Longworth DL. Antimicrobial resistance in Cairo, Egypt 1999: A survey of five hospitals. J Antimicrob Chemother. 2003;51:625–630.
25. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P, Dogra, J. Incidence of metallo beta-lactamases producing *Klebsiella pneumoniae* in ICU patients. Ind J Med Res. 2008;127:398-402.
26. Saderi H, Karimi Z, Owlia P, Bahar MA, Rad SM. Phenotypic detection of metallo

beta – lactamase producing *Klebsiella* strains isolated from burned patients. Iran J Pathol. 2008;3:20-24.

27. Ogbolu DO, Webber MA. High-level and novel mechanisms of carbapenem resistance in Gram-negative bacteria from tertiary hospitals in Nigeria. Int J Antimicrob Agents. 2014;43:412-417.
28. Magiorakos AP, Srinivasan A, Carey BB, Carmeli Y, Falagas ME, Giske CG. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18: 268–281.
29. Lowy FD. Antimicrobial resistance: The example of Staphylococcus aureus. J Clin Invest. 2003;111:1265-1273.
30. Kaur DC, Chate SS. Study of antibiotic resistance pattern in methicillin resistant *Staphylococcus aureus* with special reference to newer antibiotic. J Glob Infect Dis. 2015;7:78-84.
31. Deans SG, Noble RC, Hiltunen R, Wuryani W, Penzes LG. Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L.) Merr and Perry: Impact upon bacteria, fungi and fatty acids levels in ageing mice. Flavour Frag J. 1995; 10:323-328.
32. Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother. 2001;47:565-573.
33. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC Complement Altern Med. 2006;6:39-48.
34. Burt S. Essential oils: Their antibacterial properties and potential applications in foods. A review. Int J Food Microbiol. 2004;94:223-253.
35. Nevas M, Korhonen AR, Lindström M, Turkki P, Korkeala H. Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. J Food Protect. 2004;67:199-202.
36. Preuss HG, Echard B, Enig M, Brook I, Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for Gram-positive and Gram-negative bacteria. Mol Cell Biochem. 2005; 272:29-34.
37. El-Shenawy MA, Baghdadi HH, El-Hosseiny LS. Antibacterial activity of plants essential oils against some epidemiologically relevant food-borne pathogens. TOPHJ. 2015;8:30-24.
38. Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett Appl Microbiol. 2008;247:174–179.
39. Stapleton PD, Shah S, Anderson JC, Kara Y, Hamilton-Miller JM, Taylor PW. Modulation of beta-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int J Antimicrob Agents. 2004; 23:462-467.
40. Jeon YJ, Park J, Kim SK. Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohyd Polym. 2001; 44:71-76.
41. No HK, Park NY, Lee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int J Food Microbiol. 2002;74: 65-72.
42. Dutta PK, Tripath S, Mehrotra GK, Dutta J. Perspectives for chitosan based antimicrobial films in food applications. Food Chem. 2009;114:1173-1182.
43. Simunek J, Tishchenko G, Hodrová B, Bartonová H. Effect of chitosan on the growth of human colonic bacteria. Folia Microbiol. 2006;51:306-308.
44. Sarasam AR, Brown P, Khajotia SS, Dmytryk JJ, Madihally SV. Antibacterial activity of chitosan- based matrices on oral pathogens. J Mater Sci Mater Med. 2008; 9:1083-1090.
45. Eldin MS, Soliman EA, Hashem AI, Tamer TM. Antibacterial activity of chitosan chemically modified with new technique. Trends Biomater Artif Organs. 2008;22: 121-133.
46. Andres Y, Giraud L, Gerente C, Le Cloirec, P. Antibacterial effects of chitosan powder: Mechanisms of action. Environ Technol. 2007;28:1357-1363.
47. Islam M, Masum S, Mahbub KR, Haqu Z. Antibacterial activity of crab-chitosan against *Staphylococcus aureus* and *Escherichia coli*. J Adv Sci Res. 2011; 2:63-66.
48. Tareq A, Alam M, Raza S, Sarwar T, Fardous Z, Chowdhury A, et al. Comparative study of antibacterial activity of chitin and chemically treated chitosan prepared from shrimp (*Macrobrachium rosenbergii*) shell waste. J Virol Microbiol. 2013;9.

49. Saulenier DM, Spinler JK, Gibson GR, Versalovic J. Mechanisms of probiosis and prebiosis: Considerations for enhanced functional foods. Curr Opin Biotechnol. 2009;20:135–141.
50. Šušković J, Blazenka K, Beganović J, Pavunc AL, Habjanič K, Matosic S. Antimicrobial activity of the most important property of probiotic and starter lactic acid bacteria. Food Technol Biotechnol. 2010; 48:296–307.
51. Alakomim HL, Paananen A, Suihko ML, Helander IM, Saarela M. Weakening effect of cell permeabilizers on gram-negative bacteria causing biodeterioration. Appl Environ Microbiol. 2006;72:4695-4703.

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