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Research Article

IMPACT OF TEA TREE OIL ON BIOFILM FORMATION AND SWARMING MOTILITY BY MDR *PROTEUS MIRABILIS*

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ABSTRACT

Emergence of multi-drug resistant isolates of *P. mirabilis* and development of new resistant microbial phenotypes are growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, there is an increased demand for developing alternative strategies to conventional antibiotic therapy and to continue studies to develop new drugs, either synthetic or natural. In this study, 25 isolates of *P. mirabilis* were isolated from different clinical samples collected from different departments of Tanta University hospitals. All isolates were resistant to amoxicillin, azithromycin and tetracycline. A high incidence of resistance was also recorded for cefprozil (96%) and cefotaxime (80%). *P. mirabilis* isolates were moderately resistant to imipenem (64%), lomefloxacin (64%), ampicillin (60%), cefepime (60%) and ciprofloxacin (52%). However, the lowest incidence of resistance was recorded for amikacin (8%). All isolates showed MAR indices >0.2 and exhibited MDR profile. The antimicrobial activity of tea tree oil was evaluated using agar dilution method recording MIC50 value of 12.8 mg/ml. The effect of tea tree oil on the strong biofilm producer isolates with relatively wide swarming zone diameters was evaluated. Results obtained showed that tea tree oil (1/4 and 1/2 MIC) presented significant and dose-dependent inhibition in the biofilm production by the tested isolates of *P. mirabilis* by ≥50%. In addition, tea tree oil inhibited the swarming motility of *P. mirabilis* isolates in a dose-dependent manner. Therefore, tea tree oil could act as a potential source of alternative antimicrobials or as antipathogenic compound against MDR *P.mirabilis*.

KEYWORDS: Tea tree oil, biofilm, swarming motility, *Proteus mirabilis*

INTRODUCTION

Proteus mirabilis, a Gram-negative rod-shaped bacterium, is well-known for its urease production and distinctive ability to differentiate into elongated swarm cells and characteristic bull's-eye pattern of motility on agar plates. *P. mirabilis* belongs to the class *Gammaproteobacteria*, and has long been recognized as a member of the order *Enterobacteriales*, family *Enterobacteriaceae*. However, one group recently created a reconstructed phylogenetic tree based on shared core proteins, ribosomal proteins, and four multilocus sequence analysis proteins, and has proposed that the order *Enterobacteriales* be reclassified, placing *Proteus* within a new *Morganellaceae* family[1].While the bacterium is capable of causing a variety of human infections, including those of wounds, the eye, the gastrointestinal tract, and the urinary tract, it is most noted for infections of the catheterized urinary tract, known as catheter-associated urinary tract infections (CAUTIs)[2]. *P. mirabilis* is an agent of catheter biofilm formation, quickly fouling the surface of a newly inserted urinary catheter. Surface organelles such as fimbriae and other adhesins appear to play a significant role in this process[3]. The multidrug-resistant isolates are becoming increasingly common. Thus, efforts to generate effective vaccines or therapeutic treatments are warranted[4]. Medicinal and aromatic plants are a major source of natural organic compounds widely used in medicine. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties[5]. The plant species *Melaleuca alternifolia L.* is from the *Myrtaceae* family and has antibacterial, antifungal, and antiseptic properties[6]. Our study focused on evaluating the antimicrobial effect of tea tree oil against *P. mirabilis*, the impact of treatment with sub-MICs of tea tree oil on biofilm production and swarming motility displayed by MDR *P. mirabilis* isolates from hospitalized patients in Tanta region.

MATERIALS AND METHODS

Ethics statement

All experiments were conducted according to national and international ethical standards. The experimental protocols, including sample collection from Tanta University teaching hospitals, and consent forms, were revised and approved by the Research Ethics Committee, Faculty of Pharmacy, Tanta University, Egypt.

Bacterial isolates

A total of 25 *P. mirabilis* isolates were collected from different departments of Tanta university hospital. The clinical isolates were examined microscopically and were subjected to standard biochemical tests (the oxidase test, glucose and lactose fermentation, gas and H2S production in triple sugar iron agar, citrate utilization, motility and indole production in indole motility medium, and urease production and methyl red tests) according to the protocols suggested by MacFaddin[7].

Reference strain

Proteus mirabilis ATCC 35639 was used as quality control strain.

Chemicals

Tea tree oil (*Melaleuca alternifolia*) was obtained from Chemajet Company (Egypt) with concentration of 70 % w/v. Working solutions of tested oil were prepared in 0.5% (v/v) tween-20 to facilitate its miscibility. Other Chemicals utilized in this study were of analytical grade, obtained from Sigma-Aldrich, USA.

Antimicrobial susceptibility testing

P. mirabilis isolates were tested for susceptibility by subjection to a panel of 14 antimicrobials on Muller-Hinton agar (MHA) using agar dilution method and the results were interpreted, based on the MIC values into resistant (R), intermediate (I) or sensitive (s) isolates according to the clinical breakpoints provided by the Clinical Laboratory Standards Institute[8]. The following antimicrobials were tested (with concentrations ranged from 0.5 to 1024 µg ml^{-1}): Amoxicillin, Ampicillin, Cefprozil, Cefotaxime, Ceftazidime, Cefepime, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Lomefloxacin, Levofloxacin, Azithromycin, Tetracycline.

Multiple antibiotic resistance (MAR) index study and multi-drug resistance (MDR) character analysis

Based on the resistance patterns of the isolates, the MAR indices of the isolates were calculated and noted[9] according to the following equation:

MAR index = Number of antibiotics to which the isolate was resistant / Total number of antibiotics to which the isolate was subjected

As reported by Magiorakos[10], the isolate that showed resistance to at least one agent in three or more antimicrobial categories is considered multidrug-resistant (MDR). The isolate that showed resistance to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) is considered of extensively drug resistance (XDR) profile. Pan drug resistant (PDR) isolates are those showed resistance to all agents in all antimicrobial categories.

Determination of minimum inhibitory concentration (MIC) of essential oils

The agar dilution method recommended by CLSI (2018) was used with the following modification; a final concentration ranged between 0.2 - 25.6 mg ml^{-1} of test oil was adjusted in MHA medium containing 0.5 % v/v tween-20 to enhance oil miscibility. Plates were dried at room temperature for 30 minutes prior to spot inoculation with 3 µl aliquots of culture containing approximately 10^5 CFU ml^{-1}. Inoculated plates were incubated at 37°C for 18 h and the MIC was determined. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing test oil was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each isolate on the agar plate[11].

Effect of sub-MICs of tea tree oil on biofilm formation and swarming motility by *P. mirabilis*

The inoculum for all assays was prepared from MDR *P. mirabilis* isolates previously treated with different concentrations of test oil (1/8, 1/4, 1/2 MIC) and compared with the control (bacterial isolates untreated with tea tree oil). The treatment of bacterial isolates with test oil was done as described by Sanchez-Torrez[12]. Each test was performed in triplicate and the results represented the average of at least three independent experiments.

Biofilm assay

The test isolates were screened first for biofilm formation using the crystal violet assay[13]. Each assay was performed three times and the results were averaged. Values of absorbance ≥ 0.12 were regarded as bioflm positive, < 0.2 were considered weak producers, 0.2-0.4 were moderate producers, and > 0.4 were considered strong producers[14]. The effect of test oil on the biofilm formation in the strong biofilm producers was investigated using microtitre plate assay (crystal violet assay)[13,15]. Briefly, overnight cultures of tested isolates were added into 1 mL of fresh LB medium in the presence and the absence of sub-MICs of test oil. Bacteria were allowed to adhere and grow without agitation for 24 hours at 30 ºC. After incubation, microtitre plate was emptied by removing the media along with free-floating planktonic cells and the wells were gently rinsed twice with sterile water. The surface-attached cells (biofilm) were stained with 200 µL of 0.1% crystal violet solution. After 15 min, CV solution was discarded completely and wells were filled with 200 µL of 33% glacial acetic acid to solubilize CV from the stained cells. The biofilm biomass was then quantified by measuring the absorbance at OD 570 nm in a microplate reader.

Motility assay

Swarming motility assays were performed based on a previously described method[16]. Briefly, overnight untreated and oil treated cultures of *P. mirabilis* isolates were point inoculated onto swarm agar plates containing glucose (1%), bactoagar (0.5%), bactopeptone (0.6%), and yeast extract (0.2%). The plates were incubated at 37°C for 24 h and after incubation the diameter of swarming zone was measured in millimeters (mm).

Statistical analysis

Descriptive statistics used to describe numerical variables using mean and standard deviation to compare between control and different concentrations were done using one-way ANOVA to allow for detection of significance on biofilm formation and swarming motility after treatment with different concentrations of tea tree oil. Differences were considered statistically significant at P-value < 0.05.

RESULTS AND DISCUSSION

Different clinical samples were collected from different departments of Tanta University teaching hospitals, including; tracheal secretion, sputum, urine, stool, burn and wound swabs. All these biological samples were cultured on MacConkey agar. The non-lactose fermenting colonies on MacConkey agar were selected and subjected to a panel of standard biochemical tests. Results indicated the identification of *P. mirabilis*. Twenty five isolates of *P. mirabilis* were randomly selected for further studies. Clinical sources of the selected 25 isolates are shown in Table 1. All the selected bacterial isolates were subjected to susceptibility testing against different antimicrobials using agar dilution method. The incidence of resistance of the selected *P. mirabilis* (Fig. 1) against the tested antimicrobials ranged between 8 and 100%. All isolates were resistant to amoxicillin, azithromycin and tetracycline. Additionally, a high incidence of resistance was recorded for cefprozil (96%) and cefotaxime (80%). However, *P. mirabilis* isolates were moderately resistant to imipenem (64%), lomefloxacin (64%), ampicillin (60%), cefepime (60%) and ciprofloxacin (52%). On the other hand, the lowest incidence of resistance was recovered for amikacin (8%). Similar results of the anti-microbial susceptibility testing were recorded by Kang *et al.* where 69.44% of the isolates were resistant to ampicillin and only 5.56% of the isolates were resistant to amikacin[17].

Table 1: Clinical sources of the selected 25 *P. mirabilis* isolates

Clinical source	Distribution of the selected bacterial isolates among different clinical samples n (%)*
Tracheal secretions	2 (8)
Sputum	1 (4)
Urine	18 (72)
Stool	-
Burn	2 (8)
Wound	2 (8)
Total	25 (100)

Table 2: Antimicrobial resistance patterns and multiple antibiotic resistance (MAR) indices of *P. mirabilis* isolates

Antimicrobial resistance patterns		Pattern incidence	Bacterial isolates	MAR index	Resistance profile
Pattern code	**Resistance marker ***				
Pr I a	AMX-CPR-AZI-TET	1	Pr 4	**0.29**	MDR
Pr II a	AMX-CIP-LOM-CTX-AZI-TET	1	Pr 8	**0.43**	MDR
Pr II b	AMX-CPR-CIP-FEP-AZI-TET	1	Pr 11		MDR
Pr III a	AMX-CPR- CIP -LEV- CTX -AZI-TET	1	Pr 19	**0.5**	MDR
Pr III b	AMX-AMP-CPR- CIP -IPM-AZI-TET	1	Pr 9		MDR
Pr III c	AMX-AMP-CPR- CIP - CTX -AZI-TET	2	Pr 5, 12		MDR
Pr IV a	AMX-CPR- CIP -GN- LOM - CTX -AZI-TET	1	Pr 13	**0.57**	MDR
Pr IV b	AMX-AMP-CPR- CIP - LOM - CTX -AZI-TET	1	Pr 17		MDR
Pr IV c	AMX-CPR-CAZ-FEP-IPM-GN-AZI-TET	1	Pr 24		MDR
Pr IV d	AMX-CPR-FEP-IPM-GN- CTX -AZI-TET	1	Pr 25		MDR
Pr V a	AMX-CPR- CIP -CAZ-GN- LOM - CTX -AZI-TET	1	Pr 3	**0.64**	MDR
Pr V b	AMX-AMP-CPR- CIP -FEP-IPM-GN-AZI-TET	1	Pr 10		MDR
Pr V c	AMX-CPR-FEP-IPM-LEV- LOM - CTX -AZI-TET	1	Pr 14		MDR
Pr VI a	AMX-AMP-CPR-FEP-IPM- LEV -LEV- CTX -AZI-TET	1	Pr 1	**0.71**	MDR
Pr VI b	AMX-CPR- CIP -CAZ-FEP-IPM-AK- CTX -AZI-TET	1	Pr 18		MDR
Pr VI c	AMX-AMP-CPR- CIP -IPM- LEV - LOM - CTX -AZI-TET	1	Pr 16		MDR
Pr VI d	AMX-AMP-CPR-CAZ-FEP-IPM- LOM - CTX -AZI-TET	1	Pr 7		MDR
Pr VI e	AMX-AMP-CPR- CIP -FEP-IPM- LOM - CTX -AZI-TET	1	Pr 20		MDR
Pr VI f	AMX-AMP-CPR-FEP-AK- LEV - LOM - CTX -AZI-TET	1	Pr 22		MDR
Pr VII a	AMX-AMP-CPR-CAZ-FEP-IPM-GN- LOM - CTX -AZI-TET	1	Pr 23	**0.79**	MDR
Pr VII b	AMX-AMP-CPR-CAZ-FEP-IPM- LEV - LOM - CTX -AZI-TET	3	Pr 2, 6, 21		MDR
Pr VIII a	AMX-AMP-CPR-CAZ-FEP-IPM-AK-GN- LOM - CTX -AZI-TET	1	Pr 15	**0.86**	MDR

*__AMX__; Amoxicillin, **AMP**; Ampicillin, **CPR**; Cefprozil, **CTX**; Cefotaxime, **CAZ**; Ceftazidime, **FEP**; Cefepime, **IPM**; Imipenem, **AK**; Amikacin, **GN**; Gentamicin, **CIP**; Ciprofloxacin, **LOM**; Lomefloxacin, **TET**; Tetracycline, **LEV**; Levofloxacin, **AZI**; Azithromycin

Table 3: Effect of sub-MICs of tea tree oil on biofilm formation by selected bacterial isolates

Bacterial isolates	% reduction in biofilm production at		
	1/8 MIC	**1/4MIC**	1/2 MIC
Pr 23	20	53	63
Pr 7	40	58	63
Pr 16	35	60	71
Pr 15	49	55	61
Pr 20	43	52	57
Pr 21	35	56	60
Pr 22	46	58	64

Table 4: Percentage reduction in swarming zone diameter by sub-MICs of tea tree oil against the selected *P. mirabilis* isolates

Bacterial isolates	% reduction in swarm zone diameter at		
	1/8 MIC	**1/4MIC**	1/2 MIC
Pr 23	20	40	43
Pr 7	20	48	52
Pr 16	5	40	45
Pr 15	8	32	36
Pr 20	13	52	57
Pr 21	5	35	40
Pr 22	6	39	44

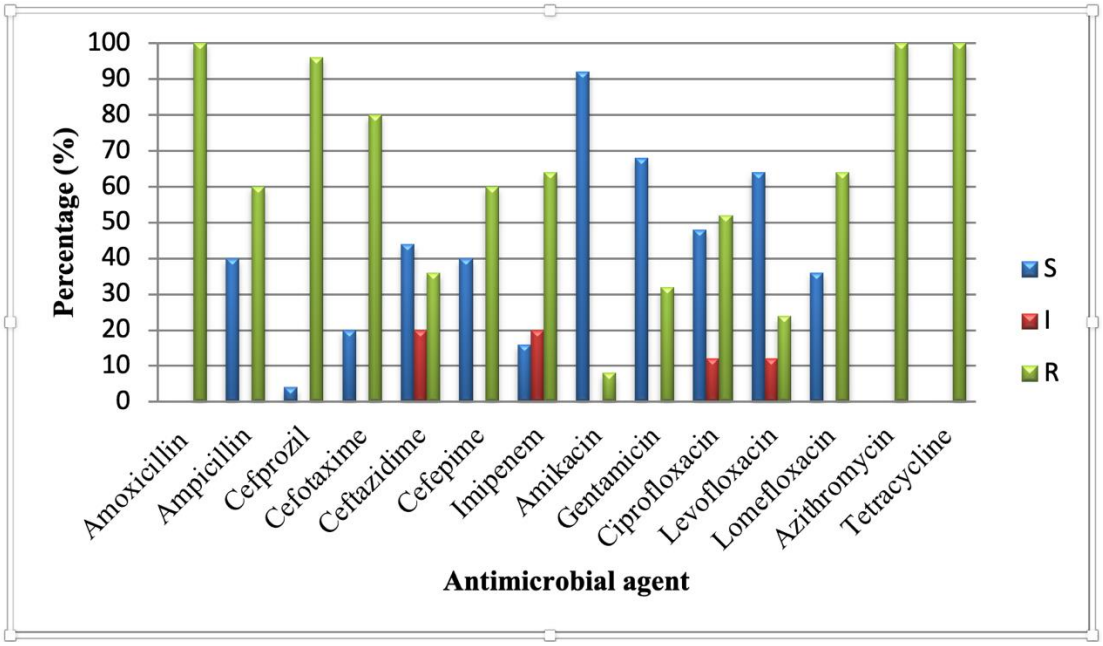

Figure 1: Incidence of resistance of selected *P. mirabilis* isolates to different antimicrobials

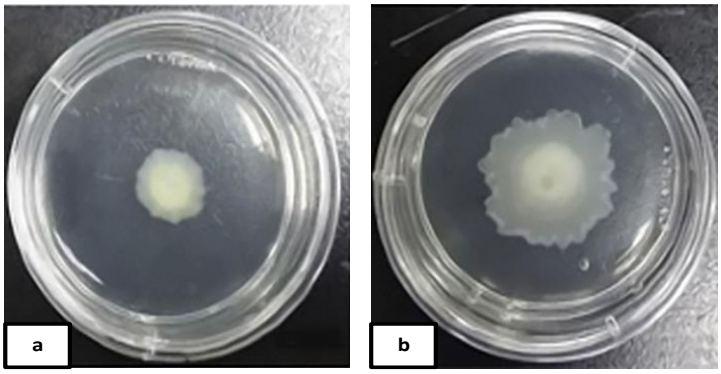

Figure 2: Reduction in swarming zone by 1/2 MIC of tea tree oil in *P. mirabilis* (Pr20) (a) compared to control (b).

Girlich *et al*. reported that higher levels of resistance to imipenem commonly occur in *P. mirabilis* isolates consecutively to the loss of porins, reduced expression of penicillin binding proteins (PBPs) PBP1a, PBP2, or acquisition of several antibiotic resistance genes, including carbapenemase genes[18]. In addition, resistance to fluoroquinolones is also frequently reported due to the frequent use in treating UTI infections[18]. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria[11]. Hence, MAR index and multi-drug resistance profile character were studied in our tested isolates. Resistance patterns and Multiple Antibiotic Resistance (MAR) indices of all tested isolates against the studied antimicrobial drugs are shown in Table 2. Multiple antibiotic resistance (MAR) index is helpful in analyzing health risk, and is used to check the antibiotic resistance, also it is considered as a good tool for risk assessment to give an idea of the number of bacteria showing antibiotic resistance in the risk zone in the routine susceptibility testing. If the MAR index value is more than 0.2, this means that isolates were originated from the environment where antibiotics were over used[19]. *P. mirabilis* tested isolates showed MAR index values between 0.29 and 0.79 and only one isolate had a value of 0.86. These results mean that these resistant isolates would have originated from environments where antibiotics were over used. Based on the resistance patterns of the isolates, the MDR, XDR and PDR characters are specified. Fortunately, none of the tested isolates showed PDR profile. On the other hand, all (100%) of *P. mirabilis* isolates exhibited MDR profile. Pal *et al*. recorded relatively similar results where more than 95% of *P. mirabilis* isolates were found to be multiple drug resistant (MDR) and none of them were pan drug-resistant (PDR)[20]. The cost of antimicrobial resistance is immense, both economically as well as for human health and lives. Therefore, research efforts aiming to identify new antimicrobial strategies are highly demanded[21]. As revealed by various recent studies, plant-based antimicrobials are promising agents fighting against drug resistant human pathogens, as revealed by various recent studies. Among the plant-derived products, essential oils (EOs) are considered the most diverse classes of specialized metabolites that play an important role in the plant defensive response to microbial. Their potent and broad spectrum antimicrobial activities have generated impressive reports in the medical literature [22,23,24]. The antimicrobial activity of tea tree oil against the tested isolates of *P. mirabilis* were determined using the agar dilution method. Results revealed the susceptibility to tea tree in the range of (12.8-25.6 mg/ml) (MIC50=12.8 mg/ml). Kulkarni *et al.* reported approximately similar results[25]. The use of some essential oils at sub-inhibitory concentrations targeted the bacterial virulence hence impairing processes that are required for a bacterium to establish an infection and cause a disease without placing direct life-or-death pressure on the organism. Targeting

virulence in this way would help to preserve the many symbioses between the microorganism and host that contribute to human health, but that are radically disrupted by traditional antibacterial therapy[26]. The test isolates were screened for biofilm production and the swarming motility. Seven strong biofilm producers (OD > 0.4) with relatively wide swarming zone diameters ranged between 18-30 mm were selected to evaluate the effect of tea tree oil. The results obtained showed that tea tree oil (1/4 and 1/2 MIC) presented significant and dose-dependent inhibition in the biofilm production of the tested isolates of *P. mirabilis* by ≥ 50% ($p< 0.05$) (Table 3). The effect of tea tree oil on the swarming motility revealed that, tea tree oil inhibited the swarming motility of *P. mirabilis* isolates in a dose-dependent manner (Table 4). Furthermore, using 1/2 MIC of tea tree oil exerted significant percentage of reduction (57%) ($p< 0.05$) in the swarming motility of *P. mirabilis* (Pr20) (Fig. 2). Similarly, Agha reported that tea tree oil demonstrated a potent anti-swarming and antibiofilm activity against *P. mirabilis* where there was complete biofilm eradication[27.] Terpinen-4-ol is a major component of TTO and has been considered the main antimicrobial component of the oil[28].

CONCLUSION

The findings of this study demonstrated an increase in the resistance to a number of antibiotics at an alarming level. This increased resistance of *P. mirabilis* is a matter of concern globally. Hence continuous monitoring of antibiotic susceptibility testing should be made mandatory to improve the empirical treatment. In light of this, a paradigm shift in the treatment of *P. mirabilis* is necessary to prevent antibiotics becoming obsolete, and where appropriate, alternative to antibiotic ought to be considered. The present study confirmed the potential antimicrobial properties of tea tree oil against *P. mirabilis*. Additionally, tea tree oil , at sub-MIC values could act as powerful anti-pathogenic drug that not, per se, inhibit growth but instead interfere directly with microbial activity. Tea tree oil significantly reduced the biofilm production and the swarming motility by *P. mirabilis*. This concept is highly attractive because it is unlikely to pose a selective pressure for the development of resistance. However, data regarding toxicity and safety upon administration is mandatory. It is also imperative to establish whether the *in vitro* results of tea tree oil translated *in vivo* into similar outcomes.

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REFERENCES

1. Adeolu M, Alnajar S, Naushad S, Gupta SR. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology.* 2016;66:5575–5599.
2. Armbruster CE, Prenovost K, Mobley HLT, Mody L. How Often Do Clinically Diagnosed Catheter-Associated Urinary Tract Infections in Nursing Home Residents Meet Standardized Criteria? *J Am Geriatr Soc.* 2016.
3. Armbruster CE, Smith SN, Johnson AO, DeOrnellas V, Eaton KA, Yep A, Mody L, *et al*. The Pathogenic Potential of *Proteus mirabilis* is Enhanced by Other Uropathogens During Polymicrobial Urinary Tract Infection. *Infection and Immunity.* 2017;85:e00808–00816.
4. Armbruster CE, Mobley HLT, Pearson MM. Pathogenesis of *Proteus mirabilis* infection. *EcoSal Plus* [Internet]. 2018;8(1).
5. Shaaban HA. Essential Oil as Antimicrobial Agents: Efficacy, Stability, and Safety Issues for Food Application. In 2020.
6. Amri I, Mancini E, De Martino L, Marandino A, Lamia H, Mohsen H, *et al*. Chemical composition and biological activities of the essential oils from three *Melaleuca* species grown in Tunisia. *Int J Mol Sci*. 2012;13(12):16580–91.
7. MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria, Williams and Wilkins. Philadelphia, PA; 2000.
8. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute. Wayne; 2018.
9. Subramani S, Vignesh S. MAR index study and MDR character analysis of a few golden staph isolates. *Asian Journal of Pharmacy and Life Science ISSN*. 2012;2231:4423.
10. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*. 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(3):268–81.
11. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. *BMC Complement Altern Med*. 2006;6(1):39.
12. Sanchez-Torres V, Hu H, Wood TK. GGDEF proteins YeaI, YedQ, and YfiN reduce early biofilm formation and swimming motility in *Escherichia coli*. *Appl Microbiol Biotechnol*. 2011;90(2):651–8.
13. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, *et al*. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol*. 1985;22(6):996–1006.
14. Piechota M, Kot B, Frankowska-Maciejewska A, Grużewska A, Woźniak-Kosek A. Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. *Biomed Res Int*. 2018;2018:4657396.
15. O'Toole GA, Pratt LA, Watnick PI, Newman DK, Weaver VB, Kolter R. [6] Genetic approaches to study of biofilms. *Methods in Enzymology*. 1999;310:91–109.
16. Kalia M, Yadav VK, Singh PK, Sharma D, Pandey H, Narvi SS, et al. Effect of cinnamon oil on quorum sensing-controlled virulence factors and biofilm formation in *Pseudomonas aeruginosa*. *PLoS One*. 2015;10(8):e0135495.
17. Kang Q, Wang X, Zhao J, Liu Z, Ji F, Chang H, *et al*. Multidrug-Resistant *Proteus mirabilis* isolates carrying *blaOXA-1* and *blaNDM-1* from wildlife in China: Increasing public health risk. *Integr Zool* [Internet]. 2020;(1749-4877.12510).
18. Girlich D, Bonnin RA, Dortet L, Naas T. Genetics of Acquired Antibiotic Resistance Genes in Proteus spp. *Front Microbiol*. 2020;11:256.
19. Paul S, Bezbaruah RL, Roy MK, Ghosh AC. Multiple antibiotic resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*. *Lett Appl Microbiol*. 1997;24(3):169–71.
20. Pal N, Sharma N, Sharma R, Hooja S, Maheshwari RK. Prevalence of multidrug (MDR) and extensively drug resistant (XDR) Proteus species in a tertiary care hospital, India. *Int J Curr Microbiol Appl Sci*. 2014;3:243–52.

21. Serwecińska L. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water (Basel)*. 2020;12(12):3313.
22. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials-A review. *Plants*. 2017;6(2):16.
23. Subramani R, Narayanasamy M, Feussner K-D. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. 3 *Biotech* [Internet]. 2017;7(3).
24. Gradinaru AC, Aprotosoaie AC, Trifan A, Spac A, Brebu M, Miron A. Interactions between cardamom essential oil and conventional antibiotics against *Staphylococcus aureus* clinical isolates. *Farmacia*. 2018;62(6):1214–1222.
25. Kulkarni A. Monitoring of antimicrobial effect of GC-MS standardized *Melaleuca alternifolia* oil (tea tree oil) on multidrug resistant uropathogens. *IOSR J Pharm Biol Sci*. 2012;2(2):6–14.
26. Rhind JP. Essential Oils (Fully Revised and Updated 3rd Edition): A Comprehensive Handbook for Aromatic Therapy. *Singing Dragon*; 2019.
27. Agha YY. Effect of some plant oils on swarming motility and Biofilm formation in Proteus, Aeromonas, and Pseudomonas. *Journal of Advanced Pharmacy Education & Research*| Oct-Dec. 2020;10(4):65.
28. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 2006;19(1):50–62.

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