Multidrug and Pan-Antibiotic Resistance—The Role of Antimicrobial and Synergistic Essential Oils: A Review

Karen Boren1, AliceAnn Crown1, and Richard Carlson1

Abstract

Bacterial resistance to antibiotics continues to be a grave threat to human health. Because antibiotics are no longer a lucrative market for pharmaceutical companies, the development of new antibiotics has slowed to a crawl. The World Health Organization reported that the 8 new bacterial agents approved since July 2017 had limited clinical benefits. While a cohort of biopharmaceutical companies recently announced plans to develop 2-4 new antibiotics by 2030, we needn’t wait a decade to find innovative antibiotic candidates. Essential oils (EOs) have long been known as antibacterial agents with wide-ranging arsenals. Many are able to penetrate the bacterial membrane and may also be effective against bacterial defenses such as biofilms, efflux pumps, and quorum sensing. EOs have been documented to fight drug-resistant bacteria alone and/or combined with antibiotics. This review will summarize research showing the significant role of EOs as nonconventional regimens against the worldwide spread of antibiotic-resistant pathogens. The authors conducted a 4-year search of the US National Library of Medicine (PubMed) for relevant EO studies against methicillin-resistant Staphylococcus aureus, multidrug-resistant (MDR) Escherichia coli, EO combinations/synergy with antibiotics, against MDR fungal infections, showing the ability to permeate bacterial membranes, and against the bacterial defenses listed above. EOs are readily available and are a needed addition to the arsenal against resistant pathogens.

Keywords

bioactivity, essential oil, combination/synergy, biofilm, efflux pump, quorum sensing, bacterial cell membrane, MRSA

Received: July 17th, 2020; Accepted: September 9th, 2020.

The antibiotic era began in 1941 with the welcomed release of penicillin. However, Alexander Fleming, who discovered penicillin, warned that microbes are educated to resist penicillin. The Centers for Disease Control and Prevention (CDC) reported that bacterial resistance to penicillin developed just 1 year later in 1942. Antibacterial resistance has continued to evolve, assisted by the overuse of antibiotics in humans, and antibiotics used to increase food animal growth in factory farming. Ultimately, 2015 brought news that Pan-resistance to antibiotics used for Gram-negative bacteria had arrived with colistin resistance that developed in China.

Researchers from Chinese, British, and US universities published in Lancet Infectious Diseases their discovery of a new form of resistance to the antibiotic of last resort, colistin. The initial colistin resistance gene is called mcr-1. Since this resistance presumably came from massive agricultural use of colistin in China, National Geographic headlined the news story “Apocalypse Pig.” Before ending its use of colistin in animals at the end of April 2017, China had been using more than 8000 metric tons of colistin per year. By 2019, there were 9 mobilized mcr genes identified (mcr-1-mcr-9).

The German Center for Infection Research reported on the plasmid-mediated colistin resistance mechanism mcr-1 in an article entitled “Dangerous and ‘jumping’ mcr-1 resistance gene.” In studying the transferability of the easily transferred gene, they stated that the “mcr-1 gene not only exists on mobile plasmids, but [can] be integrated into chromosomes as well. Consequently, it can be reliably passed on to next generations.”

The “jumping” plasmid mcr-1 indeed jumped. Klebsiella pneumoniae, Shigella flexneri, Acinetobacter baumannii, Escherichia coli, and Pseudomonas aeruginosa are also now resistant to all known antibiotics.

1Young Living Essential Oils, UT, USA

Corresponding Author:
Richard Carlson, Young Living Essential Oils, UT 84043, USA.
Email: richcarlson@youngliving.com
Research from the ensuing years has not been encouraging. While human-to-human spread of colistin resistance is to be expected, research has shown that even our waters are now carriers. Plasmid-mediated colistin-resistant ESBL-producing *E. coli* was retrieved from seawater at a public beach in Norway. According to the study, “This report illustrates that *E. coli* strains containing plasmid-mediated colistin resistance genes have also reached areas where this drug is hardly used at all. Surveillance of colistin resistance in environmental, veterinary, and human strains is warranted also in countries where colistin resistance is rare in clinical settings.”

A colistin-resistant ESBL-producing *Enterobacteriaceae* was isolated from river water and imported vegetable samples in Switzerland. Sewage in Barcelona, Spain, carried mcr-1 *Enterobacteriaceae.* Colistin-resistant mcr-1 *E. coli* was found on 2 Brazilian beaches, including Santos, the major beachfront city of the region with the largest shipping terminal in Latin America. The colistin resistance gene was found in 18 different locations on the Haihe River in China. ESBL, KPC-type, and mcr-1.2-producing *Enterobacteriaceae* contaminated wells, river water, and wastewater treatment plants in Northern Italy, while a pan-drug-resistant isolate of *A. baumannii* was recovered in municipal waste water in Zagreb, Croatia. Finally, we note a September 2019 study published in *Infection Control & Hospital Epidemiology.* Henig et al report using real-time polymerase chain reaction to identify 4 patients with the mobile colistin resistance mcr-1 gene in a hospital in Michigan.

With extensive drug resistance and pan resistance now worldwide, microbial transfer has taken on fearful new capabilities. A February 2017 study in *Nature Microbiology* linked flies for the first time to the spread of carbapenem resistance. The researchers reported: “Furthermore, local *blan* dissemination (flies and dogs) increases… the probability of carriage by migratory birds such as swallows, the migratory winter destinations of which are usually South East Asia (including Cambodia, Vietnam, Laos, Thailand, Malaysia and Indonesia).” A 2018 Sichuan University concurred stating “the movable colistin-resistance gene *mer-1* was detected among the colistin-resistant *E. coli* strains isolated from the river water and egret feces, which indicated the possibility of the environmental dissemination of this gene.”

### The Multiple Mechanisms of Essential Oils

Schnaubelt discussed the antibacterial efficacy of essential oils (EOs): “Unlike antibiotics, which are active due to inhibiting an easily identifiable single target, the activity of EOs, impairing a bacterium in multiple physiological systems as well as in membrane functionality, is only now understood.”

A 2019 study lists some of the antibacterial pathways of EOs: “The most frequently reported mechanism of antibacterial action of both isomers [carvacrol and thymol] involves the disruption of bacterial membrane leading to bacterial lysis and leakage of intracellular contents… Other proposed mechanisms of antibacterial action include the inhibition of efflux pumps, prevention in the formation and disruption of preformed biofilms, inhibition of bacterial motility, and inhibition of membrane ATPases.”

A lesser-known aspect of EOs is while powerful against pathogenic bacteria, some studies suggest they have little effect on beneficial bacteria. An abstract presented at the Experimental Biology 2019 meeting reported: “Using cinnamon essential oil as an antibiotic alternative has shown to be a promising option with limited detrimental effects against the commensal bacteria.” Lead author Victoria R. Adams said further evaluation was needed to determine the benefits of utilizing a natural alternative.

A 2009 study showed “*Carum carvi* [caraway], *Lavandula angustifolia* Mill. [lavender], *Trachyspermum copticum* [ajowan], and *Citrus aurantium* var. *amara* [neroli] essential oils displayed the greatest degree of selectivity, inhibiting the growth of potential pathogens at concentrations that had no effect on the beneficial bacteria examined.” Si et al reported, “Thymol, cinnamon oil and carvacrol have previously demonstrated a broad spectrum of antimicrobial activities… Our results not only confirmed the activity of these compounds against *E. coli* and *Salmonella,* but also demonstrated their selectivity towards the pathogens with little effect on the beneficial gut bacteria.” Also, a 2015 study in *Microbiology* reported that “thymol and geraniol at around 100 ppm could be effective in suppressing pathogens in the small intestine, with no concern for beneficial commensal colonic bacteria in the distal gut.”

Abreu et al explain that "plants, rather than relying on single metabolites, use a combination of strategies and a highly efficient defense system that includes very diverse molecules ranging from proteins to H₂O₂ and oxygen radicals, which most likely complement each other, to deal with microbial threats. The defense strategies may thus involve the synergistic activity of 2 or more compounds, which could act via different mechanisms and/or targets… It is important to bear in mind that millions of years of evolution have resulted in plant defense systems that have proved to be not readily susceptible to microbial resistance mechanisms.”

### EOs Against Methicillin-Resistant *Staphylococcus aureus*

A 2019 case study and literature review in *Therapeutic Advances in Infections Disease* reported that in “2013, the Centers for Disease Control and Prevention (CDC) highlighted methicillin-resistant *Staphylococcus aureus* (MRSA) as a serious drug-resistant threat citing over 80,000 invasive MRSA infections leading to over 11,000 deaths each year in the United States. MRSA frequently enters the blood stream and metastasizes resulting in endocarditis and osteomyelitis. This represents a major burden on the healthcare system, with 30- and 90-day mortality rates as high as 30% and 50%
Deaths in the United States from antibiotic-resistant infections are more than 35,000 yearly. In the study mentioned above, Abreu et al. identified plant extract compounds that could act as antibiotic adjuvants “showing their great potential for application in the clinical therapy of infections with antibiotic-resistant microorganisms such as MRSA.”

A review by Chambers and Deleo noted CA MRSA has characteristics not found in HA MRSA. Tissue-destructive infections that include necrotizing fasciitis and fulminant, necrotizing pneumonia may be a result of CA MRSA genes that encode the virulence factor Panton-Valentine Leukocidin, which induces leukocyte destruction and tissue necrosis. Please see Table 1 for more substantiated data.

EO/Antibiotic Additivity & Synergism

Rosato et al. reported peppermint EO/gentamicin and ampicillin synergism. The “results against Gram-negative bacteria [such as K. pneumoniae and Pseudomonas aeruginosa are of particular interest as these bacteria are difficult to treat with commonly employed antibiotic drugs.”

The 2016 review by Aelenei et al. noted that the intrinsic difference between Gram-positive and the more-difficult-to-kill Gram-negative bacteria is “due to an additional outer membrane acting as an effective barrier for amphipathic agents and over expression of efflux pumps responsible for innate antimicrobial resistance.” The authors concluded that their review “reveals that essential oils and their purified components enhance the efficacy of antibiotics against Gram-negative bacteria, being promising candidates for the development of new effective formulations against Gram-negative bacteria.”

In their research against MDR Pseudomonas aeruginosa, Utchariyakiat et al. found “cinnamon bark oil showed the strongest antimicrobial activity against all clinical-isolated MDR-PA strains with MIC of 0.0562 to 0.225% v/v and MBC of 0.1125 to 1.8% v/v.” This study also reported that “cinnamon bark oil and cinnamaldehyde combined with colistin demonstrated synergistic rates at 16.7% and 10%, respectively.” Please see Table 2 for more substantiated data.

Escherichia coli: 2 Devasting Clone-Sequence Types: ST 405 and 1193

Gram-negative E. coli has increased in virulence. In 2016 in New Jersey, 2 mobile genes in E. coli were found to carry the mcr-1 and blaNDM-5 genes conferring resistance to colistin and carbapenems. Co-author in this study, Barry Kreiswirth later stated: “The bad news is that... there are clearly other strains out there that haven’t detected yet. Both the carbapenem resistance and the colistin resistance genes are on separate plasmids, which means in principle they could spread to other bacteria.”

Tchesnokova et al. discovered in 2019 a new MDR E. coli affecting younger adults (under age 40) E. coli clonal group, sequence type 1193 emerging in multiple US cities. This MDR-E. coli is 100% resistant to fluoroquinolones, 55% resistant to trimethoprim-sulfamethoxazole, and 53% resistant to tetracycline. The multipronged effects of EOs may provide a boost to antibiotics that are now ineffective for MDR E. coli. Please see Table 3 for more substantiated data.

Candida auris: The Deadly MDR Fungi Hospitals Would Not Disclose

April 2017 news reports told of an outbreak of pan-resistant Candida auris in New York, New Jersey, and Illinois. One deadly case was at Northwestern Memorial Hospital in Chicago contracted by a patient from a catheter or intravenous line. It has been reported that nearly half the people who contract this die within 90 days. There was no announcement to the community about the outbreak.

This nonaction followed a model of suppression set by Royal Brompton Hospital outside London. Only after the intensive care unit at Royal Brompton was shut down for 50 Candida auris cases did they acknowledge the outbreak. Patients were moved to another floor for 11 days.

A newspaper article told how London hospital workers used a device to spray aerosolized hydrogen peroxide throughout the room of a patient with Candida auris. After a week’s treatment the “settle plate” used to confirm bacterial efficacy showed just 1 organism grew back: Candida auris.

The 2016 European C. auris outbreak was discussed by Schelzen et al. who reported, “Despite a comprehensive review of modern technologies for environmental decontamination there is currently no published data in the literature on the effectiveness of cleaning agents or decontamination of the environment for C. auris specifically.”

In 2018, China had its first cases of pan-echinocandin-resistant Candida tropicalis and pan-echinocandin-resistant Candida glabrata, which suggests monitoring for antifungal susceptibility trends in all Candida species.

We note a 2014 study in Acta Biochem Pol. as just one of many showing antifungal effects of EOs. Budzyńska et al. state: “This report includes the results proving the influence of clove oil, geranium oil, lemon balm and citronella oil on possible mechanisms reported to be relevant for Candida pathogenesis, namely germ tube and mycelium formation, adhesive and invasive properties and extracellular production of various enzymes.” Please see Table 4 for more substantiated data.

EO Effects on Bacterial Cell Membrane

A 2013 Italian study described EO mechanisms of action against microbes. “Toxic effects on membrane structures and function are generally used to explain the antimicrobial activity of EOs. In fact, the mechanisms of action of the EOs include the degradation of the cell wall, damaging the membrane proteins, increased permeability leading to leakage of the cell contents, reducing the proton motive force, reducing the intracellular ATP pool via decreased ATP synthesis and augmented..."
| Table 1. EO/Constituent Against MRSA |
|-------------------------------------|
| **EO/Constituent**                  | **Method**                          | **Bioactivity**               | **Critical finding**                                                                 | **Ref.** |
| Nigella sativa L. / EO/carvacrol,  | Broth microdilution/PCR/             | Membrane integrity           | Results show components p-cymene, thymoquinone, and carvacrol disrupted bacterial    | 24       |
| thymoquinone, p-cymene              | biofilm formation assay              |                             | membrane of the MRSA strain.                                                        |          |
| Origanum vulgare L., carvacrol,     | Agar diffusion method                | Antimicrobial                | The MIC for carvacol: 0.015%-0.03%, v/v; thymol: 0.03-0.06, v/v; oregano oil:        | 25       |
| thymol                              |                             |                             | 0.06%-0.125%, v/v. Oregano oil shows potential as a topical antibacterial               |          |
| CPV orange oil Citrus sinensis, L.  | Disc diffusion/agar diffusion        | Cell lysis                   | The CPV orange oil showed inhibition and bactericidal effect on MRSA as well          | 26       |
| Osbeck                              |                             |                             | as vancomycin intermediate-resistant S. aureus strains in the in vitro model.          |          |
| Eugenol/citral from Syzygium        | Agar dilution, microdilution,       | Antimicrobial                | Sequential exposure of S. aureus L. mononogynates strains to eugenol or citral did   | 27       |
| aromaticum (L.) Merr. & L.M. Perry  | crystal violet assay                |                             | not result in development of resistance to the oil component itself or to antibiotics |          |
| 91 EOs/EO blends were tested.       | Disc diffusion assay                | Antimicrobial                | The concentration of each oil or blend tested against MRSA was 30 µL. While           | 28       |
| Including Cymbopogon flexuosus      |                             |                             | 78 oils had measurable inhibitory activity, the Cymbopogon flexuosus ZOI was            |          |
| (Nees ex Steud.) WWatson/R.C. blend |                             |                             | >83 mm as was the R.C. blend's. The authors state that both Cymbopogon flexuosus and    |          |
| Eucalyptus globulus Labill, Myrtus  |                             |                             | R.C. blend inhibited all MRSA growth on the plate (not noting any role vapor might     |          |
| communis L., Origanum majorana     |                             |                             | play).                                                                                |          |
| L., Pinus sylvestris L., E. radiata |                             |                             |                                                                                     |          |
| A. Cunn ex DC., E. citriodora Hook, |                             |                             |                                                                                     |          |
| Lavandula angustifolia Mill,        |                             |                             |                                                                                     |          |
| Cupressus sempervirens L., Picea    |                             |                             |                                                                                     |          |
| mariana Mill./Britton, Sterns &     |                             |                             |                                                                                     |          |
| Poggenb., Mentha × piperita L.      |                             |                             |                                                                                     |          |
| Melaleuca alternifolia (Maiden &    | Disc diffusion/modified broth       | Antimicrobial                | All 60 MRSA isolates tested against tea tree were susceptible. There was no           | 29       |
| Betchel) Cheel                      | microdilution                     |                             | difference in susceptibility between mupirocin-resistant/sensitive S. aureus.         |          |
| Chamomyparts obtusa                 | MIC: serial dilution method        | Antimicrobial                | Universal susceptibility to tea tree oil of all MRSA and MSSA isolates tested        | 30       |
| (Sielbold & Zucc.) Endl.            |                             |                             | represents a significant result in the application for MRSA control.                  |          |
| Melaleuca alternifolia (Maiden &    | In vivo                           | Antimicrobial                | Effective against MDR MRSA/VRE as well as M. luteus ATCC 9341, M. smegmatis ATCC     | 31       |
| Betchel) Cheel                      |                             |                             | 9341, Pseudomonas aeruginosa KCTC 1637, and E. faecalis ATCC 29212.                    |          |
| Tea tree Melaleuca                  | PCR detection of SA442 and         | Anti-biofilm                 | While treatment with mupirocin 2% nasal ointment and a tea tree regimen had           | 32       |
| alternifolia (Maiden & Betchel)     | MecA genes/disk diffusion          |                             | similar results, tea tree may be useful as an alternative to mupirocin in areas of    |          |
| Cheel                               | assay                             |                             | high mupirocin resistance.                                                           |          |
| Eucalyptus varieties: E. globulus   | Broth microdilution                | Antimicrobial                | All Eucalyptus varieties were active against MRSA NCTC 10422 (and 9 other              | 33       |
| Labill. (fruit and leaf), E.        |                             |                             | strains), but it was E. globulus (fruit) oil that showed the best activity (MIC         |          |
| radiata A. Cunn. Ex DC., E.         |                             |                             | between 250 and 1000 µg/mL). This strong showing could be attributed to               |          |
| citriodora Hook, (leaf)             |                             |                             | aromadendrene's lipophilic ability to disrupt the MRSA biomembrane.                   |          |
| Anethum graveolens L. (Dill EO)     | BALB/c mice model/RT-PCR analysis  | Antimicrobial                | Dill EO shortens the MRSA inflammatory stage and induces apoptosis by promoting p53    | 34       |
| Aromadendrene/1,8-cineole from      |                             |                             | and caspase-3 expression.                                                            |          |
| Eucalyptus globulus Labill. (fruit) |                             |                             | Aromadendrene apparently contributes significantly to the antimicrobial activity of   | 35       |
| |                                |                             |                             | EGF. Combinations of aromadendrene and 1,8-cineole showed additive effects in most    |          |
| |                                |                             |                             | cases, but also synergistic behavior in the time-kill assay. EGF exhibited a         |          |
| |                                |                             |                             | pronounced antimicrobial effect toward MDR bacteria.                                 |          |

(Continued)
Table 1. Continued

| EO/Constituent       | Method                                      | Bioactivity | Critical finding                                                                                                                                                                                                 | Ref.  |
|----------------------|---------------------------------------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| *Piper cubeba* L.    | Transmission electronic microscope/atomic force microscopy | Antimicrobial | EOs from *P. cubeba* L. show activity as an antibacterial agent against methicillin- and oxacillin-resistant *S. aureus*, acting on the bacterial cell wall and plasma membrane.                                  | 36    |
| *Lavandula coronopifolia* Poir. | Broth micro-well dilution | Antimicrobial | *L. coronopifolia* was found to be effective against MRSA with ZOI (mm) of 16.0 ± 1.00.                                                                                                                        | 37    |
| *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson | Disc diffusion, MBIC, MBEC | Antimicrobial | Lemongrass and citral both completely cleared the plate of bacterial growth w/ ZOI of >8.60 cm. Demonstrating lemongrass EO possesses anti-biofilm activity at low concentrations. | 38    |
| *Tagetes minuta* L. | Disc diffusion test | Antimicrobial | EO of *Tagetes minuta* showed a high activity against MRSA with an inhibition zone of 23 mm.                                                                                                              | 39    |
| *Melaleuca alternifolia* (Maiden & Betchel) Cheel | MIC/MBC | Antimicrobial | Results might provide the basis for a possible role of TTO as part of nonconventional regimens against both MSSA/MRSA and Gram-negative MDR/PDR microorganisms.                                               | 40    |
| *Schinus arorara* L. | Broth microdilution assay | Antibacterial | Antibacterial effectiveness could be synergistic effects of α-phellandrene and limonene or disruption of bacterial membrane integrity.                                                                           | 41    |
| *Pogostemon cablin* (Blanco) Benth./pogostone | In vitro/in vivo Kunming mice | Antibacterial | Pogostone showed antibacterial activity against MRSA with an average MIC of 400 µg/mL. The in vivo animal study against MRSA ATCC43300 showed MIC at 400.000 ± 0.000.                                            | 42    |

Abbreviations: AHLs, N-acyl homoserine lactones; CA MRSA, Community Associated Methicillin-resistant *Staphylococcus aureus*; CDR, *Candida auris* resistant; CPV, Cold-pressed Valencia; EGF, epidermal growth factor; EO, essential oil; FICI, Fractional Inhibitory Concentration; LVO, Lavender essential oil; MBC, Minimum bacterial concentration; MBEC, Minimum biofilm eradication concentration; MBIC, Minimum biofilm inhibitory concentration; MDR, multidrug-resistant; MFC, Minimum fungicidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; OSEO, *Ocimum sanctum* essential oil; PCR, polymerase chain reaction; UTIs, Urinary tract infections; ZOI, Zone of inhibition.
| EO/ Antibiotic/ Pathogen | Method | Critical finding | Ref. |
|--------------------------|--------|------------------|-----|
| *Myrtus communis* L. / polymyxin B, ciprofloxacin / Acinetobacter baumannii | Checkerboard method, time-kill curve method | Myrtle EO’s action concerns cell wall and membrane structures, changing their permeability and leading to release of intracellular contents outside of cell. | 61 |
| *Cinnamomum tamala* (Buch.-Ham)/DNase1/DNase MBD (Marine bacteria)/ *Pseudomonas aeruginosa* | MIC, crystal violet assay, scanning electron microscopy | This study shows synergism of EO *C. tamala* with DNases. This use may be an effective treatment of *P. aeruginosa* biofilm infections. | 62 |
| *Rumex officinalis* L. (REO)/neomycin, gentamicin, meronitazole, amikacin, meronidazole, benzolmetronidazole, nystatin, anphoteracin B/MDR *S. aureus*, *Escherichia coli* and *Candida* strains *albicans* and *knesi* | Broth microdilution | Evidence was shown of the modifying-antibiotic resistance activity of REO when tested in combination with aminoglycoside against MDR *S. aureus*, *E. coli*. Although REO sensitized the *C. krusei* strain to *S. aureus* B/MDR, *Escherichia coli* oxacillin, novobiocin/ESBL- | 63 |
| *Lavandula angustifolia* Mill./Chloramphenicol, Ciprofloxacin, Fusidic acid, Nystatin/ *C. albicans*, *S. aureus*, *Pseudomonas aeruginosa* | Microdilution assay | Lavender EO has potential to improve antibacterial efficacy of some antibiotics. EOs affect many antibacterial pathways and combined with antibiotics offer possible potentiation through synergistic effects. | 64 |
| *Pétanthes chloreonana* (Coss. & Durieu) Schinz, *Tocenium ramossissimum* Desf. and *Pistacia lentiscus* L./ amoxicillin, tetracycline, piperacillin, ofloxacin, oxacillin, novobion/ESBL- | MTT, assay in vitro cytotoxic assay, time-kill assay, broth microdilution checkerboard method | The data support the potential of natural antimicrobials to enhance antibiotics actions toward clinical MDR bacteria. This suggests the potential use of these oils with pharmaceutical products, diminishing harmful side effects and treatment costs of the synthetic drugs. | 65 |
| Lavender EO/Octenidine dihydrochloride | MIC, time- killing curves, FTIR analysis | Based on results of time-killing curve it may be assumed that LEO has a synergistic effect on OCT, thereby enhancing its permeation into bacterial cells. | 66 |
| *Origanum vulgare* L., *Thymus vulgaris* L., *Lavandula angustifolia* Mill., *Mentha piperita*, and *Melaleuca alternifolia* (Maiden & Betchel) Cheil/ *antibacterials: gentamicin, ampicillin, antifungal: gentamicin, miconazole, *S. typhimurium*, fungals: *Candida* spp. *S. aureus*, *S. mutans*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida* spp. *C. glabrata*, *C. krusei*, *C. kefyr* | Checkerboard microdilution method | Synergism of carvacrol and erythromycin supports reuse of erythromycin against GAS strains. | 67 |
| Nine EOs, including *Mentha × piperita* L. and *Carum carvi* L. (caraway) with gentamicin against ESBL-producing and NDM-producing *K. pneumoniae* isolates | Checkerboard method | With *M. piperita* the MIC for gentamicin is markedly reduced, more than 30-fold lower against 6 out of the 10 bacteria strains considered. Strong synergy between gentamicin and peppermint against Gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* is much lower than normally required. | 68 |
| *Mentha × piperita* L./antibacterials: gentamicin, ampicillin, antifungal: amphothecin B, FLC, miconazole/ *B. cereus*, *B. subtilis*, *Staphylococcus* spp., *A. baumannii*, *Pseudomonas aeruginosa*. Fungals: *Candida* spp. *C. guillemontii*, *C. glabrata*, *C. krusei*, *C. kefyr* | MIC, EtBr accumulation assays | All tested compounds found to present synergistic effect with NA against *S. typhimurium*, thymol most effective. | 69 |
| Nine EOs, including *Mentha × piperita* L. and *Carum carvi* L. (caraway) with gentamicin against ESBL-producing and NDM-producing *K. pneumoniae* isolates | Checkerboard method | Synergism of carvacrol and erythromycin supports reuse of erythromycin against GAS strains. | 67 |
| *Mentha × piperita* L./FLC/ *Candida* spp. | MIC, Checkerboard assay, scanning electron microscopy | The combination of bio-AgNP with OEO resulted in synergistic and additive antimicrobial activities against the MDR bacterial strains of *E. coli*, *A. baumannii* and MRSA. | 71 |
| EO/Antibiotic/Pathogen | Method | Critical finding |
|-----------------------|--------|------------------|
| Ocimum basilicum L., Salvia edward L., Rosmarinus officinalis L. (out of 8 oils tested)/gentamicin, ciprofloxacin, amikacin, netilmicin, tobramycin, chloramphenicol, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, cefoxitin, erythromycin, clindamycin, rifampicin, linezolid, fusidic acid, quinupristin/dalfopristin, kanamycin, mupirocin, vancomycin | Disc diffusion assay, MIC, MBC | Lipophilic nature of monoterpenes causes expansion of the membrane, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes. Additive and synergistic effects of the 3 oils with antibiotics were noted. |
| Cinnamomum cassia (L.) J Presl./streptomycin, ampicillin, chloramphenicol/MDR E. coli, Staphylococcus aureus, P. aeruginosa | Agar disc diffusion, MIC, checkerboard assay | Antibiotics with cinnamon EO reduces MICs 2-fold for Pseudomonas aeruginosa, 2-8-fold for S. aureus, and 2-4-fold for E. coli. There were synergistic effects of cinnamon with ampicillin or chloramphenicol against S. aureus, and by combination EO and chloramphenicol against E. coli. |
| Citrus hystrix DC. EO/chlorhexidine/P. gingivalis, S. mutans, S. sanguinis | Time-kill assay, checkerboard microdilution assay | Bacterial cell membrane disrupted at 4× MIC for 4 hours; at 4× MIC cell membrane completely destroyed at 8 hours. |
| Cinnamomum verum J. Presl./meropenem/P. pneumoniae | Time-kill assay CLSI guideline; checkerboard assay | Study showed that additivity and synergistic interaction between CBO and antibiotic were comparable in ability to cause bacterial membrane disruption. |
| Paired combinations piperacillin and Cinnamomum verum J.Presl; piperacillin & Lavandula angustifolia Mill; piperacillin & Mentha × piperita L.; meropenem & Mentha × piperita L/beta-lactamase-producing E. coli | MIC, Checkerboard assay | Two- to four-fold reduction in the MIC of the antibiotics was observed in some paired combinations: piperacillin/cinnamon bark and piperacillin and lavender. |
| Nepeta nuda L. ssp. nuda/1,8 cineole, tetracycline, and streptomycin/E. coli, K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus | Microdilution checkerboard assay, chemoinformatics method | N. nuda oil-antibiotic and 1,8-cineole- antibiotic resulted in antagonistic interactions. Chemoinformatics confirms antagonistic interactions via membrane dissipation. |
| Satureja kitaibelii Wierzb. ex Heuff, ex Heuff, geraniol/tetracycline, chloramphenicol/E. coli, K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus | Microdilution checkerboard assay, chemometric method | Synergistic interactions of EO and antibiotic; constituent and antibiotic, reduced MIC values against Gram-negative bacteria. |
| Libanotis montana Grant subsp. leucocarpa (Heuff) Soó/tetracycline, streptomycin, chloramphenicol/E. coli, K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus | Microdilution checkerboard assay, chemometric method | Synergistic or additive combinations reduced the minimum effective antibiotic dose, which minimized side effects. Strong synergy, especially against Gram-negative bacteria, was noted. |

Abbreviations: EO, essential oil; FLC, fluconazole; FTIR, Fourier transform infrared; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus; NDM, New Delhi metallo-β-lactamase-1.
Table 3. EOs Against *Escherichia coli* and MDR-*E. coli*.

| Essential oil/Constituent/Pathogen | Method | Critical finding | Ref. |
|-----------------------------------|--------|-----------------|------|
| *Eucalyptus globulus* Labill., *Cinnamomum verum* J.Presl, *Rosa marinus officinalis* L., *Daucus carota* L., and *Camelina sativa* /*E. coli* | Broth microdilution | Two blends of EOs were effective against antibiotic-resistant ESBL-*E. coli* and MRSA among others. | 48 |
| *Origanum compactum* Benth./*E. coli* | MIC, MBC | Irreversible damage on the cell wall and membrane was caused by *O. compactum* EO leading to the leakage of proteins and genetic materials (DNA and RNA). | 49 |
| *Origanum majorana* L., *Thymus zygis* L., and *Rosmarinus officinalis* L./*E. coli* | Disc diffusion, MIC, MBC | This synergistic combination showed 16-fold sensitivity against MDR-*E. coli*, which is normally insensitive to vancomycin. | 50 |
| *Oregano and thyme red oils* /carvacrol and thymol | Crystal-violet static formulation assay, CLSM/COMSTAT analysis | Components compromised *E. coli* cytoplasmic membrane integrity causing intracellular potassium leakage. | 51 |
| *Cinnamomum zeylanicum* Blume, *Syzygium aromaticum* Merr. & L.M. Perry, *Cymbopogon flexuosus* (Nees ex Steud.)WWatson, *Origanum vulgare* Steud./*E. coli* | Time-kill assay | This study tested both Gram-positive and -negative pathogens. | 52 |
| *Litsea cubeba* (Lour.) Pers. testing | Disc diffusion protocol | When cinnamon bark EO was tested 19 times (*n* = 19) against ESBL-*E. coli*, the mean kill zone diameter was 29.7 mm with an SD of 0.82 mm. The median and range of the kill zone were 30 and 3 mm, respectively. | 53 |
| *Achillea wilhelmsii* K. Koch, *Echinophra platyloba* D.C., *Leptospermum scoparium* J.R.Forst. & G.Forst./*E. coli* | Disc diffusion, agar diffusion | Oregano and thyme red oils/carvacrol and thymol exhibited high biofilm activity; reduced virulence of UPEC by limiting ability to agglutinate erythrocytes and to survive in human whole blood. | 54 |
| *Satureja intermedia* C.A.Mey./*E. coli* | Crystal-violet static formulation assay, PCR | O. *nigra* EO was found effective against nosocomial ESBL-*E. coli* isolates with 2 methods. | 55 |
| *L. reuteri* (Lour.) Pers. testing 1,8-cineole (LC19) and linalool (BV27) types | MIC, MBC, time kill | *E. coli* was selected as the model strain to investigate mechanism of action of the 2 types. BV27 (linalool) led to cell permeabilization, which also changes in nucleoid morphology indicating disruption of membrane integrity. | 56 |
| *L. myrrha* and *P. vulgaris* had great antibacterial activity against ESBL-producing *E. coli* isolates. *A. wilhelmsii*, *E. platyloba*, *S. nemorosa*, as did *S. intermedia*. | MIC, double-disc synergy test, PCR | This study tested both Gram-positive and -negative pathogens. | 57 |

Abbreviations: EO, essential oil; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.
Table 4. EOs Against MDR Fungal Infections.

| EOs                        | Pathogen          | Method                                      | Mode of action                                         | Critical finding                                                                 | Ref. |
|----------------------------|-------------------|---------------------------------------------|--------------------------------------------------------|----------------------------------------------------------------------------------|------|
| Mentha × piperita L.       | Candida albicans, C. tropicalis, C. glabrata | Broth dilution method/checkerboard assay/scanning electron microscopy | Disrupts cell membrane/target ergosterol biosynthesis pathway | Mint EO and compounds penetrate the cell membrane and target ergosterol biosynthesis pathway, thus impairing its biosynthesis. | 68   |
| Melaleuca alternifolia (Maiden & Betchel) Cheel | Flucon-resistant candidiasis in AIDS patients | In vitro susceptibility studies, MIC | Antifungal | Six of 12 patients improved, while an additional 2 were clinically cured. | 85   |
| Melaleuca alternifolia EO with methylecellulose hydrogel | C. albicans | Disc diffusion method, time-kill assay | Anti-Candida action | The antifungal properties of the hydrogels were confirmed by both zone of inhibition method and time-kill assays and the 2% (v/v) hydrogel significantly reduced the retention of C. albicans. | 86   |
| Eucalyptus uniflora L.     | C. albicans, C. krusei, C. tropicalis | Broth microdilution assay/MFC | Anti-Candida action | EOs inhibited the growth of C. albicans filamentous structures at all concentrations. The oil was inefficient against C. krusei. | 87   |
| Thymus capitatus (L.) Hoffm & Link, Rosmarinus officinalis L., Myrtus communis L., Salvia officinalis L., Myrtus communis/Pistacia lentiscus L., Pelargonium granatum L’Héér, Eucalyptus globulus L’Drill, | Variety of Candida strains | MIC/checkerboard technique, time-kill study | Broad-spectrum activity against pathogenic Candida strains | C. verum and cinnamaldehyde equally inhibited C. species growth. C. verum, P. granatum, C. ammi, and T. capitatus were potent against Candida while P. granatum and C. verum showed synergistic effects with flucytosine against fluconazole-resistant strains of C. albicans. | 88   |
| Thymol/carvacrol monoterpenes | C. albicans | Checkerboard microdilution assay, time-kill curves | Synergy w/flucytosine | Thymol and carvacrol augment the efficacy of flucytosine by chemosensitizing fungal cells to the drug and decreasing its efflux by CDR1 and MDR1 efflux pumps. These monoterpenes could be useful in antifungal chemotherapy. | 89   |
| Myrtus communis L., Zingiber officinalis Roscoe, Matricaria chamomilla L., Thymus vulgaris L., Mentha piperita L., | Fluconazole-resistant C. albicans | Disc diffusion method, MIC, MFC | Antifungal | O. vulgare and T. ammi EOs showed high antifungal activity. Interestingly, differences on susceptibility between groups of C. albicans were observed, indicating that the FLU-resistant group was more susceptible to these EOs than the FLU-susceptible group. Both oregano and T. ammi oils are high in thymol, which may explain their antifungal activity. | 90   |
| Cinnamaldehyde             | Fluconazole-resistant clinical isolates | Disc diffusion halo assay, time-kill curves | Antifungal | Candida isolates showed susceptibility to cinnamaldehyde and inhibition of H+ ATPase-mediated proton pumping. The study showed that cinnamaldehyde exhibits fungicidal but not fungistatic activity. | 91   |
| Origanum vulgare L., Cinnamomum zeylanicum Blume, Lippia graveolens Kunth, Thymus vulgaris L., Salvia officinalis L., Ruta graveolens L., Ocimum basilicum L., Zingiber officinalis Roscoe | Fluconazole-resistant and -sensitive C. glabrata | Broth microdilution, MIC, MFC | Antifungal, anti-efflux pump | C. glabrata resistance to azoles results from efflux of antifungal agents. Oregano EO showed the lowest MIC and MFC values against both the fluconazole-susceptible and -resistant isolates. Mexican oregano (Lippia graveolens) showed MICs (μg/mL) of 400 to >3200 for C. glabrata fluconazole-resistant and -sensitive isolates. | 92   |
| EO-AC’s thymol, carvacrol, citral | Cryptoccus neoformans, C. laurentii | Scanning electron microscopy, confocal laser scanning | Antifungal | Thymol best inhibited Cryptococcus sp. planktonic cells, biofilm formation, and mature biofilms, followed by carvacrol and citral. The biofilm of C. laurentii was more susceptible to EO-ACS in comparison to C. neoformans showing species-specific and drug-specific differences. | 93   |
| Pinus cembrae L., Origanum vulgare L., Thymus vulgaris L., components/itraconazole | Cryptococcus neoformans strains | Checkerboard assays, time-kill studies | Antifungal | Pine oils MIC showed the highest inhibitory activity: 0.07-0.27 mg/mL. Oregano was the most effective against the azole nonsusceptible isolate. Synergistic and additive effects were shown with combinations of pine, oregano, and thyme red with itraconazole. | 94   |
EO Effect on Biofilms/Efflux Pumps/Quorum Sensing

In 2013, Bjarnsholt reported on the “category of chronic infections caused by bacteria growing in slime-enclosed aggregates known as biofilms. Biofilm infections, such as pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis media and implant- and catheter-associated infections, affect millions of people in the developed world each year and many deaths as a consequence.”

Wang et al noted that “The increasing multidrug resistance has become a major threat to the public health. Overexpression of multidrug efflux pumps is one of the major mechanisms of drug resistance in bacteria.”

Defoirdt stated: “Many important plant, animal, and human pathogens regulate virulence by quorum sensing, bacterial cell-to-cell communication with small signal molecules.”

EOs and their components have multifaceted effects against these powerful bacterial defenses. We recommend the 2019 review “Anti-quorum Sensing and Antimicrobial Effect of Mediterranean Plant Essential Oils Against Phytopathogenic Bacteria” by Camele et al. Please see Table 6 for more substantiated data.

MDR and XDR Tuberculosis

It is important to include EO impact on the major health problem, MDR and extensively drug-resistant (XDR) strains of tuberculosis, which affect millions. The US CDC reports that tuberculosis is one of the world’s deadliest diseases with one-fourth of the world’s population infected with it. According to the World Health Organization (WHO): “A total of 1.5 million people died from TB in 2018. Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. WHO estimates that there were 484,000 new cases with resistance to rifampicin—the most effective first-line drug, of which 785 had MDR-TB.” Of the 10 million individuals who became ill with TB in 2018, approximately three million were “missed” by health systems and do not get the care they need, allowing the disease to continue to be transmitted.

The 2018 study by Kazemian et al noted the lengthy treatment time for TB, hepatotoxicity of drugs, and the emergence of multidrug-resistance and extreme-drug-resistant strains. Their research on medicinal plants of the Lamiaceae family showed MDR-TB was completely inhibited by Zataria multiflora Boiss. at 78 µg/mL while Satureja rochbigleri and Satureja khuzestanica showed MICs of 156 µg/mL against MDR Mycobacterium tuberculosis.

Other EOs showing efficacy in studies against MDR- and XDR-TB are Myrtus communis L. and Nigella damascena.
Table 5. EO Effects on Bacterial Cell Membrane.

| EO/Bacteria                                      | Method                                      | Critical finding                                                                 | Ref. |
|--------------------------------------------------|---------------------------------------------|----------------------------------------------------------------------------------|------|
| Ocimum sanctum/60 clinical, 5 standard laboratory isolates of Candida | MIC, MFC, confocal scanning laser microscopy, sterol quantitation method         | OSEO and the major components, methyl chavicol and linalool, effectively target the cytoplasmic membrane in Candida. This action may originate with inhibition of ergosterol biosynthesis. | 101  |
| Thymus maroccanus Ball/Escherichia coli, Enterobacter aerogenes, Salmonella enterica Typhimurium | Measuring optical density, MIC and MIC/2, β-lactamase assay                       | Thymus maroccanus was shown to permeabilize both the outer and inner membranes of the bacteria tested allowing the release of proteins such as β-lactamase, β-galactosidase, or Effu. | 102  |
| Monarda punctata L. EO/Streptococcus pyogenes, MRSA, Streptococcus pneumoniae, Haemophilus influenzae, E. coli | Broth microdilution method, time-kill dynamic curves, SEM                        | Monarda punctata EO was the most successful with S. pyogenes, E. coli, and S. pneumonia with the lowest MIC and MBC values. The most resistant bacterial strain was MRSA. The oil’s major compounds are thymol, p-cymene, and limonene. In testing, the whole EO was found to be more potent than the individual compounds. | 103  |
| EO components: citronellol, citronellal, carveol, and carvone/E. coli, Staphylococcus aureus | MIC and MBC/membrane integrity: propidium iodide uptake                           | While all compounds showed antibacterial activity against E. coli and S. aureus, carvone lacked inhibitory action against S. aureus. Generally, citronellol was the most effective compound. The EO compounds’ antibacterial activity resulted in membrane/cell wall permeabilization. | 54   |
| Juniperus rigida Siebold & Zucc. /Klebsiella pneumoniae | MIC and MBC, growth curve                  | The EO caused irreversible damage to the bacterial cell wall and membrane leading to leakage of proteins. With 61 components to the oil there may be more than 1 antibacterial mechanism. | 104  |
| Lavandula angustifolia Mill./E. coli              | Time-kill assay, SEM, anti-QS assay         | Lavender EO is able to disrupt bacterial membranes of Gram-negative bacteria. When combined with antibiotics lavender increases penetration of the antibiotic into the bacteria. | 105  |
| Lavandula angustifolia Mill. EO/K. pneumoniae     | MIC, checkerboard assay, influx/efflux assay, SEM                                  | Our study showed LVO is a promising antimicrobial extract, which can be used in combinatorial therapy and may possibly be effective in revising the efficacy of carbapenem and other antibiotics against other resistant bacteria. We propose the overall mode of action of LVO: disrupting the bacterial membrane via oxidative stress. | 106  |
| Isodon melissoides (Benth.) H. Haro/12 pathogenic bacteria including MRSA, AAA-44, MRSE | MIC, protein leakage assays              | Isodon melissoides showed significant antibacterial activity against 11 bacterial strains out of 12 tested by damaging the cell membrane and changing cell membrane permeability. | 107  |
| Origanum compactum Benth.                         | MIC, MBC                                  | The bacterial cell membrane was damaged by OCEO measured by release of cell contents. | 108  |

Abbreviations: EO, essential oil; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus; SEM, scanning electron microscopy.
| EO/Constituent/Pathogen | Method | Critical finding | Ref. |
|------------------------|--------|------------------|------|
| **Biofilm**            |        |                  |      |
| Black pepper (Piper nigrum L.), cananga (Cananga odorata Lam. Hook.f. & Thomson), and myrrh (Commiphora myrrha [Nees] Engl.) EOs, and the constituent cis-nerolidol | Crystal violet assay, confocal laser microscopy, SEM assay | Black pepper, cananga, and myrrh oils and their common constituent cis-nerolidol at 0.01% markedly inhibited *S. aureus* biofilm formation. Confocal laser microscopy was used to observe biofilm formation on plate bottoms where this inhibition was observed. | 108 |
| Oregano (Origanum vulgare L.), red thyme (as neither is listed, it is assumed *Thymus vulgaris* L. as oregano contains little thymol), EO, thymol, carvacrol | SEM | Thymol and oregano EO have a better result on antibiofilm formation of *E. coli* than carvacrol. Carvacrol and thymol have a better result on anti-biofilm formation of *Salmonella* than oregano EO. | 109 |
| Artemisia vestita Wall. Ex Besser | Micro-well dilution method, SEM | The EO *Artemisia vestita* and its major component, grandisol, offer potential for development as a novel antibacterial drug for the treatment of respiratory infections, particularly against *Streptococcus pneumoniae*. | 110 |
| Of 11 EOs, most effective: Cinnamomum cassia L. J. Presl. and *Salvia officinalis* L. | MIC, MBC, MBIC, MBEC against *S. aureus* | Three microemulsions: *C. cassia*, *S. officinalis*, or both caused a >3 logarithmic reduction in *S. aureus* 24-h-old biofilms and desiccated biofilms; and up to 68% of biofilm removal after 90 min of exposure. | 111 |
| Lemongrass *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson, grapefruit (*Citrus paradisi* Macfad), EO, citral | Disc diffusion method, SEM, MIC, MBC, MBEC | Lemongrass and citral prevented biofilm formation at 0.06% (v/v) for hospital MSSA strains/0.125% for other strains tested. It did not remove already formed biofilms. No antibiofilm activity shown for grapefruit EO. | 112 |
| **Efflux pump**         |        |                  |      |
| Thymol and carvacrol from thyme (*Thymus vulgare* L.) EO | Checkerboard microdilution method | Both thymol and carvacrol decreased expression of CDR and MDR genes, which are known to encode for the efflux pumps and contribute to FLC resistance. | 113 |
| Rosmarinus officinalis L. (EO) and main constituent 1,8-cineole | Adapted diffusion method, serial binary microdilution, flow cytometry assessment | *R. officinalis* L. and 1,8-cineole exhibited very good antimicrobial activity against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* MDR strains and a synergistic activity with ciprofloxacin by inducing cellular wall permeabilization and efflux pumps inhibitory activity. | 114 |
| Thymol and carvacrol | MIC, MBC | Thymol and carvacrol inhibited the ethidium bromide (EtBr) cell efflux in a concentration-dependent manner. | 115 |
| Chenopodium ambrosioides L. and α-terpinene | MIC, efflux pump inhibition by MIC reduction | α-terpinene was not relevant in this study despite being the major compound of the oil. The oils’ success is possibly from blocking the protein source for the MFS efflux pump in the SA-1199B strain combined with destabilizing the cell membrane. | 116 |
| Chenopodium ambrosioides L. and α-terpinene | Microdilution method | The expected power of α-terpinene was not seen. Components in lower concentrations may be responsible for the oils’ ability to alter cytoplasmic membrane fluidity state resulting in destabilizing efflux pumps. Synergistic effects were seen: the oil decreased the tetracycline MIC by 2× and of EtBr by 6×. | 117 |
| *Salvia fruticosa* Mill., *Salvia officinalis* Wall, *Salvia sclarea* L. | MIC, broth checkerboard microdilution method | The FICI for the combination of tetracycline and EO *Salvia officinalis* and *S. sclarea* indicated synergistic and in some cases additive interactions. | 118 |

(Continued)
Conclusion

On April 21, 2019, a segment on CBS News asked: “Could Antibiotic-Resistant ‘Super Bugs’ Become a Bigger Killer Than Cancer?” Interviewee Ramanan Laxminarayan, senior research scholar at Princeton University, has tracked the rise of superbugs for nearly 20 years. He discussed what would not be possible without effective antibiotics. “Everything that we think of whether it’s cancer chemotherapy, transplants, hip replacements, knee replacements, colorectal surgery, all of these require effective antibiotics to perform.”

What has been the response to the antibiotic-resistance threat? “By 2006, the European Union had banned all nonmedicinal antibiotics in animals… In 2005, the emergence of fluoroquinolone-resistant *Campylobacter jejuni* in the clinical setting in conjunction with fluoroquinolone administration in animals prompted the FDA to ban fluoroquinolone use in poultry.” The authors of the study just quoted reported that following the avoparin ban in Europe, “the prevalence of VRE in farm animals rapidly declined… However, this did not translate directly to a decrease of VRE in humans.”

A systematic review and meta-analysis on reducing antibiotic use in food-producing animals was published in *Lancet Planet Health* in November 2017. The *Lancet* study concluded:

Interventions that restrict antibiotic use in food-producing animals are associated with a reduction in the presence of antibiotic-resistant bacteria in these animals. A smaller body of evidence suggests a similar association in the studied human populations, particularly those with direct exposure to food-producing animals. The implications for the general human population are less clear, given the low number of studies. The overall findings have directly informed the development of WHO guidelines on the use of antibiotics in food-producing animals.

Acting on causes of antibiotic resistance is certainly a part of solving this bacterial crisis. But foremost is research for deterrents. The synchronicity of EOs and antibiotics has been reported by Owen and Laird “as a potential solution to antibiotic resistance.” They note, however, that “a lack of consistency in methods and interpretation criteria makes drawing conclusions of efficacy of studied combinations difficult.” The multipronged effects of EOs on resistant microorganisms must be added to the antibacterial arsenal. With a paucity of new antibiotics in the pipeline, the call is now urgent for more and better designed EO/antibiotic synergy research.

Lahmar et al documented the often-surprising power of combining an EO with an antibiotic:

In the interactive experiment essential oils were found highly effective in reducing the resistance of Methicillin-resistant *Staphylococcus aureus* to amoxicillin, tetracycline, piperacillin, ofloxacin and oxacillin and resistance of *Acinetobacter baumannii* to amoxicillin and of ofloxacin in interactive manner.
Furthermore, the results proved synergism among essential oils and both antibiotics ofloxacin and novobiocin against the Extended-Spectrum Beta-Lactamase producing *E. coli* (ESBL).134

We close by recommending the 2014 review by Langeveld et al that stated: “Several modes of action have been put forward by which antibiotics and the EO components may act synergistically, such as by affecting multiple targets; by physiochemical interactions and inhibiting antibacterial-resistance mechanisms. Many reported assays show additivity or moderate synergism, indicating that EOs may offer possibilities for reducing antibiotic use.”135

**Acknowledgments**

In Memoriam: This is the 17th and final study sponsored by D. Gary Young (1949-2018).

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

**ORCID ID**

AliceAnn Crown https://orcid.org/0000-0002-9953-2001

**References**

1. Liu Y-Y, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16(2):161-168. doi:10.1016/S1473-3099(15)00424-7

2. National Geographic. https://www.nationalgeographic.com/science/phenomena/2015/11/21/mcr-gene-colistin/

3. German Center for Infection Research. Accessed June 20, 2016. https://www.dzif.de/en/dangerous-and-jumping-mcr-1-resistance-gene

4. Jorgensen SB, Soraas A, Arnesen LS, Leegaard T, Sundsfjord A, Jenum PA. First environmental sample containing plasmid-mediated colistin-resistant ESBL-producing *Escherichia coli* detected in Norway. *APMIS*. 2017;125(9):822-825. doi:10.1111/apm.12720

5. Zurfuh K, Poirel L, Nordmann P, Nüesch-Inderbinen M, Hächler H, Stephan R. Occurrence of the plasmid-borne Mer-1 Colistin resistance gene in extended-spectrum-β-lactamase-producing enterobacteriaceae in river water and imported vegetable samples in switzerland. *Antimicrob Agents Chemother.* 2016;60(4):2594-2595. doi:10.1128/AAC.00066-16

6. Ovejero CM, Delgado-Blas JF, Calero-Caceres W, Muniesa M, Gonzalez-Zorn B. Spread of mcr-1-carrying Enterobacteriaceae in sewage water from Spain. *J Antimicrob Chemother.* 2017;72(4):1050-1053.

7. Fernandes MR, Sellera FP, Esposito F, Sabino CP, Cerdeira L, Lancopan N. Colistin-resistant mcr-1-positive *Escherichia coli* in public beaches, an infectious threat emerging in recreational waters. *Antimicrob Agents Chemother.* 2017;61(7) doi:10.1128/AAC.00234-17 27 06 2017.

8. Yang D, Qiu Z, Shen Z, et al. The occurrence of the colistin resistance gene mcr-1 in the Haihe river (China). *Int J Environ Res Public Health*. 2017;14(6):576. doi:10.3390/ijerph14060576

9. Caltagirone M, Nucleo E, Spalla M, et al. Occurrence of extended spectrum β-lactamases, KPC-type, and mcr-1.2-producing *Enterobacteriaceae* from wells, river water, and wastewater treatment plants in oltrepò pavese area, Northern Italy. *Front Microbiol*. 2017;8:2232. doi:10.3389/fmicb.2017.02232

10. Goic-Batisic I, Seruga Music M, Kovacic A, Tonkic M, Hrenovic J. Pan drug-resistant environmental isolate of *Acinetobacter baumannii* from croatia. *Microb Drug Resist.* 2017;23(4):494-496. doi:10.1089/mdr.2016.0229

11. Henig O, Rojas LJ, Bachman MA, et al. Identification of four patients with colistin-resistant *Escherichia coli* containing the mobile colistin resistance mcr-1 gene from a single health system in Michigan. *Infect Control Hosp Epidemiol*. 2019;40(9):1059-1062. doi:10.1017/ice.2019.177

12. Wang Y, Zhang R, Li J, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry. *Nat Microbiol*. 2017;2(4):16260. doi:10.1038/s41564.0.260

13. Wu J, Huang Y, Yao D, Zhang Y, Yang K. Evidence for environmental dissemination of antibiotic resistance mediated by wild birds. *Front Microbiol*. 2018;9:745. doi:10.3389/fmicb.2018.00745

14. Schnaubelt K. *The Healing Intelligence of Essential Oils: The Science of Advanced Aromatherapy*. Healing Arts Press; 2011.

15. Kachur K, Suntres Z. The antibacterial properties of phenolic isomers, carvacrol and thymol. *Front Microbiol*. 2018;9:745. doi:10.3389/fmicb.2018.00745

16. FASEB. https://www.fasebj.org/doi/abs/10.1096/fasebj.2019.33.1_supplement.lb287

17. Hawrelak JA, Cattley T, Myers SP. Essential oils in the treatment of intestinal dysbiosis: a preliminary in vitro study. *Altern Med Rev*. 2009;14(4):380-384.

18. Si W, Gong J, Tsao R, et al. Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. *J Appl Microbiol*. 2006;100(2):296-305. doi:10.1111/j.1365-2672.2005.02789.x

19. Thapa D, Louis P, Losa R, Zweifel B, Wallace RJ. Essential oils have different effects on human pathogenic and commensal bacteria in mixed faecal fermentations compared with pure cultures. *Microbiology*. 2015;161(Pt 2):441-449. doi:10.1099/mic.0.000009

20. Abreu AC, Coqueiro A, Sultan AR, et al. Looking to nature for a new concept in antimicrobial treatments: isoflavonoids from...
Cytisus striatus as antibiotic adjuvants against MRSA. Sci Rep. 2017;7(1):3777. doi:10.1038/s41598-017-03716-7

21. Lewis PO, Sevinsky RE, Patel PD, Krolkowski MR, Cluck DB. Vancomycin plus nafcillin salvage for the treatment of persistent methicillin-resistant Staphylococcus aureus bacteremia following daptomycin failure: a case report and literature review. Ther Adv Infect Dis. 2018;6:2049936118797404.

22. US Centers for Disease Control. https://www.cdc.gov/drugresistance/about.html

23. Chambers HF, DeLeo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009;7(9):629-641. doi:10.1038/nrmicro2200

24. Mouwakeh A, Kincses A, Nové M, et al. Nigella sativa essential oil and its bioactive compounds as resistance modifiers against Staphylococcus aureus. Phytother Res. 2019;33(9):1101-1108.

25. Nostro A, Blanco AR, Cannatelli MA, et al. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol Lett. 2004;2:191-195.

26. Muthaiyan A, Biswas D, Crandall PG, Wilkinson BJ, Rieke SC. Application of orange essential oil as an antistaphylococcal agent in a dressing model. BMC Complement Altern Med. 2012;12:125.

27. Apolônio J, Faleiro ML, Miguel MG, Netro L. No induction of antimicrobial resistance in Staphylococcus aureus and Listeria monocytogenes during continuous exposure to eugenol and citral. FEMS Microbiol Lett. 2014;354(2):92-101.

28. Chao S, Young G, Oberg C, Nakaoka K. Inhibition of methicillin-resistant Staphylococcus aureus (MRSA) by essential oils. Flavour Fragr J. 2008;23:444-449.

29. Carson CF, Cookson BD, Farrelly HD, Riley TV. Susceptibility of methicillin-resistant Staphylococcus aureus to the essential oil of Melaleuca alternifolia. J Antimicrob Chemother. 1995;35(3):421-424.

30. Bae MS, Park DH, Choi CY, Kim KY, Yoo JC, Cho SS. Essential oils and non-volatile compounds derived from Chamaecyparis obtusa. Broad spectrum antimicrobial activity against infectious bacteria and MDR (multidrug resistant) strains. Nat Prod Commun. 2014;9(2):193-199. doi:10.1177/1934578X14004002-268. doi:10.1002/jmr.2564.10.1016/j.biopharma.2018.10.117

31. Alharbi NS, Khaled JM, Alzaharni KE, et al. Effects of Piper cebula L. essential oil on methicillin-resistant Staphylococcus aureus: an AFM and TEM study. J Mol Recognit. 2017;30(1):e2564.10.1002/jmr.2564.10.1022/jmr.2564

32. Air Said I, Zablanc K, Ghalbani I, et al. Chemical composition and antibacterial activity of Lavandula coronopifolia essential oil against antibiotic-resistant bacteria. Nat Prod Res. 2015;29(6):582-585. doi:10.1080/14786419.2014.954246

33. Adukwu EC, Allen SCH, Phillips CA. The anti-biofilm activity of lemongrass (Cymbopogon flexuosus) and grapefruit (Citrus paradisi) essential oils against five strains of Staphylococcus aureus. J Appl Microbiol. 2012;113(5):1217-1227. doi:10.1111/j.1365-2672.2012.05418.x

34. Abin NA, Sharopov FS, Al-Kaf AF, et al. Composition of essential oil from Tagetes minuta and its cytotoxic, antioxidant and antimicrobial activities. Nat Prod Commun. 2014;9(2):193-199. doi:10.1177/1934578X14004002-268. doi:10.1002/jmr.2564.10.1016/j.biopharma.2018.10.117

35. Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of Eucalyptus globulus against antibiotic-susceptible and antibiotic-resistant pathogens. Phytother Res. 2010;17(13):1061-1066. doi:10.1002/ptr.3068.

36. Aelenei P, Miron A, Trifan A, Bujor A, Căpătă A, Aprotosoaie E. 2018;23(10):2672. doi:10.3390/flux.2018.10.117

37. Chomnawang MT. Efficacy of cinnamon bark essential oil against multi-drug resistant Staphylococcus aureus. Molecules. 2018;23(10):2584. doi:10.3390/molecules23102584

38. Celaya LS, Alabrudzińska MA, Molina A, Viturro CI, Moreco S. The inhibition of methicillin-resistant Staphylococcus aureus by essential oils isolated from leaves and fruits of Schinus avera according to their chemical compositions. Acta Biotrop Pol. 2014;61(1):41-46. doi:10.18388/abp.2014_1921

39. Chen J, Chou S, Ong P, Peng F, et al. In vitro and in vivo antibacterial activity of pogostone. Chin Med J. 2014;127(23):4001-4005.

40. Rosso A, Catalano A, et al. Efficacy of the synergistic action of Mentha piperita essential oil with common antimicrobials. PLos One. 2018;13(6):e0200902. doi:10.1371/journal.pone.0200902

41. Alcântara M, Miron A, Trifan A, Bujor A, Căpătă A, Aprotosoaie E. Efficacy of cinnamon bark essential oil against multi-drug resistant Staphylococcus aureus. Molecules. 2018;23(10):2584. doi:10.3390/molecules23102584

42. Utchitaryakiat I, Surassomboon P, Khunthayanoponn, P, Chomnawang MT. Efficacy of cinnamon bark essential oil and cinnamaldehyde on anti-multidrug resistant Pseudomonas aeruginosa and the synergistic effects in combination with other antimicrobial agents. BMC Complement Altern Med. 2016;16:158. doi:10.1186/s12906-016-1134-9
46. Mediavilla JR, Patrawalla A, Chen L, et al. Colistin- and carbapenem-resistant *Escherichia coli* harboring mec-1 and bla<sub>NDM-5</sub>, causing a complicated urinary tract infection in a patient from the United States. *MBio*. 2016;7(4). doi:10.1128/mbio.01191-16

47. Tchernokova VL, Rechlin F, Larson L, et al. Rapid and extensive expansion in the united states of a new multdrug-resistant *Escherichia coli* Clonal group, sequence type 1193. *Clin Infect Dis*. 2019;68(2):334-337. doi:10.1093/cid/ciy525

49. Bouyahya A, Abrini J, Dakka N, Bakri Y. Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. *J Pharm Anal*. 2019;9(5):301-311. doi:10.1016/j.jpha.2019.03.001

50. Lagha R, Ben Abdallah F, Al-Sarhan BO, Al-Sodany Y. Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against *Escherichia coli* Isolated from UTI patients. *Molecules*. 2019;24(6):1161. doi:10.3390/molecules24061161

51. Hamoud R, Zimmermann S, Reichling J, Wink M. Synergistic interactions in two-drug and three-drug combinations (thymol, EDTA and vancomycin) against multi drug resistant bacteria including *E. coli*. *Phyto medicine*. 2014;21(4):443-447. doi:10.1016/j.phymed.2013.10.016

52. Kaskatepe B, Yildiz SS, Kiymaci ME, Yazgan AN, Cesur S, Erdem SA. Chemical composition and antimicrobial activity of the commercial *Origanum onites* L. oil against nosocomial carbapenem resistant extended spectrum beta lactamase producer *Escherichia coli* isolates. *Acta Biol. Hung*. 2017;68(4):466-476.

53. Lee JH, Kim YG. Lee J. Carvacrol-rich oregano oil and thymol-rich thyme red oil inhibit biofilm formation and the virulence of uropathogenic *Escherichia coli*. *J Appl. Microbiol*. 2017;123(6):1420-1428.

54. Lopez-Romero JC, Gonzalez-Rios H, Borges A, Simoes M. Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. *Evid Based Compliment Alternat Med*. 2015;2015:795435.

55. Mith H, Clinguart A, Zhari A, Daube G, Delcenserie V. The impact of oregano (*Origanum vulgare*) essential oil and carvacrol on virulence gene transcription by *Escherichia coli*. *FEMS Microbiol Lett*. 2015;362(1):1-7.

56. Nguyen HV, Meile J-C, Lebrun M, Caruso D, Chu-Ky S, Sarter S. Littsa cubba leaf essential oil from Vietnam: chemical diversity and its impacts on antibacterial activity. *Lett Appl Microbiol*. 2018;66(3):207-214. doi:10.1111/lam.12837

57. Sharifi-Rad J, Mneyer D, Roointan A, et al. Antibacterial activities of essential oils from Iranian medicinal plants on extended-spectrum β-lactamase-producing *Escherichia coli*. *Cell Mol Biol (NY)*. 2016;62(9):75-82.

58. Patterson JE, McElmeel L, Wiederhold NP. In vitro activity of essential oils against gram-positive and gram-negative clinical isolates, including carbapenem-resistant *Enterobacteriaceae*. *Open Forum Infect Dis*. 2019;6(12):ofz502. doi:10.1093/ofid/ofz502

59. Yang X, Sha K, Xu G, et al. Subinhibitory concentrations of allcin decrease uropathogenic *Escherichia coli* (UPEC) biofilm formation, adhesion ability, and swimming motility. *Int J Mol Sci*. 2016;17(7):979. doi:10.3390/ijms17070979 29 Jun 2016.

60. Yap PS, Krishnan T, Chan KG, Lim SH. Antibacterial mode of action of *Cinnamomum verum* bark essential oil, alone and in combination with piperacillin, against a multi-drug-resistant *Escherichia coli* strain. *J Microbiol Biotechnol*. 2015;25(8):1299-1306.

61. Aleksic V, Mimica-Dulic N, Simin N, Nedeljkovic NS, Knezevic P. Synergistic effect of *Myrtus communis* L. essential oils and conventional antibiotics against multi-drug resistant *Acinetobacter baumannii* wound isolates. *Phyto medicine*. 2014; 21(12):1666-1674. doi:10.1016/j.phymed.2014.08.013

62. Farisa Banu S, Rubini D, Rakshitaa S, et al. Antiviral properties of underexplored *Cinnamomum tamala* essential oil and its synergistic effects with DNase against *Pseudomonas aeruginosa* Biofilms - an in vitro study. *Front Microbiol*. 2017;8:1144. doi:10.3389/fmicb.2017.01144

63. Barreto HM, Silva Filho EC, Lima EDO, et al. Chemical composition and possible use as adjuvant of the antibiotic therapy of the essential oil of *Rassmarinus officinalis* L. *Ind Crops Prod*. 2014;59:290-294. doi:10.1016/j.indcrop.2014.05.026.

64. de Rapper S, Viljoen A, van Vuuren S. The *in vitro* antimicrobial effects of *Lavandula angustifolia* essential oil in combination with conventional antimicrobial agents. *Evid Based Compliment Alternat Med*. 2016;2016:1-9. doi:10.1155/2016/275279

65. Magi G, Marini E, Facinelli B. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant group A streptococci. *Front Microbiol*. 2015;6(12):165. doi:10.3389/fmicb.2015.00165

66. Miladi H, Zmantar T, Koushid B, et al. Use of carvacrol, thymol, and eugenol for biofilm eradication and resistance modifying susceptibility of *Salmonella enterica* serovar typhimurium strains to nalidixic acid. *Microb Pathog*. 2017;104:56-63. doi:10.1016/j.micpath.2017.01.012

67. Kwaitkowski P, Pruss A, Grygorczewicz B, et al. Preliminary study on the antibacterial activity of essential oils alone and in combination with gentamicin against extended-spectrum β-lactamase-producing and new delhi metallo-β-lactamase-1-producing *Klebsiella pneumoniae* isolates. *Microb Drug Resist*. 2018;24(9):1368-1375. doi:10.1016/j.mdr.2018.05.051

68. Sambor N, Khan A, Varma A, Manzoor N. Synergistic anti-candidal activity and mode of action of *Monilia piperita* essential oil and its major components. *Pharm Biol*. 2015;53(10):1496-1504. doi:10.3109/13880209.2014.989623

69. Scandoriero S, de Camargo LC, Lancheros CAC, et al. Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. *Front Microbiol*. 2016;7(699):760. doi:10.3389/fmicb.2016.00760

70. Sienkiewicz M, Lysakowska M, Kowalczyk E, et al. The ability of selected plant essential oils to enhance the action of recommended antibiotics against pathogenic wound bacteria. *Burns*. 2017;43(2):310-317. doi:10.1016/j.burns.2016.08.032
97. Khosravi AR, Minoochianhaghighi MH, Shokri H, Emami SAAsili J, Alavi SM. The potential inhibitory effect of Cuminum cyminum, ziziphus clinoiodioides and Nigella sativa essential oils on the growth of Aspergillus fumigatus and Aspergillus. Braz J Microbiol. 2011;42(2):216-224. doi:10.1590/ S1517-83822011000100027

98. Gemeda N, Woldeamanuel Y, Asrat D, Dehella A. Effect of Cymopogon martinii, Vominculum vulgare, and Tradycpernum ammi essential oils on the growth and mycotoxins production by Aspergillus species. Int J Food Sci. 2014;2014(6):1-9. doi:10.1155/2014/874135

99. Yahyaarayat R, Khosravi AR, Shahbazzadeh D, Kalaj V. The potential effects of Zataria multiflora Boiss essential oil on growth, aflatoxin production and transcription of aflatoxin biosynthesis pathway genes of toxigenic Aspergillus parasiticus. Braz J Microbiol. 2013;44(2):643-649. doi:10.1590/S1517-83822013000200045

100. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013;6(12):1451-1474. doi:10.3390/ph6121451

101. Khan A, Ahmad A, Akhtar F, et al. Osimum sanctum essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. Res Microbiol. 2010;161(10):816-823. doi:10.1016/j.resmic.2010.09.008

102. Fadli M, Chevalier J, Bolla J-M, Mezrioui N-E, Hassani L, Pages J-M. Thymus maroccanus essential oil, a monoterpene oil of drug against gram-positive bacteria and resistant isolates. J Appl Microbiol. 2012;113(5):1120-1129. doi:10.1111/j.1365-2672.2012.05401.x

103. Li H, Yang T, Li F-Y, Yao Y, Sun Z-M. Antibacterial activity and mechanism of action of Monarda punctata essential oil and its main components against common bacterial pathogens in respiratory tract. Int J Clin Exp Pathol. 2014;7(11):7389-7398.

104. Meng X, Li D, Zhou D, Wang D, Liu Q, Fan S. Chemical composition, antibacterial activity and related mechanism of the essential oil from the leaves of Juniperus rigida Sieb. et Zucc against Klebsiella pneumoniae. J Ethnopharmacol. 2016;194(16):pii:S0378-3394. [pii]. doi:10.1016/j.jep.2016.10.050

105. Yap PSX, Krishnan T, Yiap BC, Hu CP, Chan K-G, Lim SHE. Membrane disruption and anti-quorum sensing effects of synergistic interaction between Lavandula angustifolia (lavender oil) in combination with antibiotic against plasmid-conferring multi-drug-resistant Escherichia coli. J Appl Microbiol. 2014;116(5):1119-1128. doi:10.1111/jam.12444

106. Yang S-K, Yusoff K, Thomas W, et al. Lavender essential oil induces oxidative stress which modifies the bacterial membrane permeability of carbapenemase producing Klebsiella pneumoniae. Sci Rep. 2020;10(1):819. doi:10.1038/s41598-019-55601-0

107. Kumar A, Singh S, Kumar A, et al. Chemical composition, bactericidal kinetics, mechanism of action, and anti-inflammatory activity of Laurus nobilisoides (Benth.) H. Hara essential oil. Nat Prod Res. 2019;9:1-6. doi:10.1080/14786419.2019.1591399

108. Lee K, Lee J-H, Kim S-I, Cho MH, Lee J. Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against Staphylococcus aureus. Appl Microbiol Biotechnol. 2014;98(22):9447-9457. doi:10.1007/s00253-014-5903-4

109. Lee J-H, Kim Y-G, Lee J. Carvacrol-rich oregano oil and thymol-rich thyme red oil inhibit biofilm formation and the virulence of uropathogenic Escherichia coli. J Appl Microbiol. 2017;123(6):1420-1428. doi:10.1111/jam.13602

110. Yang C, Hu D-H, Feng Y. Essential oil of Artemisia vestita exhibits potent in vitro and in vivo antibacterial activity: Investigation of the effect of oil on biofilm formation, leakage of potassium ions and survival curve measurement. Mol Med Rep. 2015;12(4):5762-5770. doi:10.3892/mmr.2015.4210

111. Campana R, Casettari I, Fagioli I, Cespi M, Bonacucina G, Baffone W. Activity of essential oil-based microemulsions against Staphylococcus aureus biofilms developed on stainless steel surface in different culture media and growth conditions. Int J Food Microbiol. 2017;241:132-140. doi:10.1016/j.ifoodmicro.2016.10.021

112. Ahmad A, Khan A, Manzoor N. Reversal of efflux mediated antifungal resistance underlies synergistic activity of two monoterpenes with fluconazole. Eur J Pharm Sci. 2013;48(2):80-86. doi:10.1016/j.ejps.2012.09.016

113. Saviuc C, Gheorghe I, Coban S, et al. Rosmarinus officinalis essential oil and eucalyptol act as efflux pumps inhibitors and increase ciprofloxacin efficiency against Pseudomonas Aeruginosa and Acinetobacter Baumannii MDR strains. Rom Biotechnol Lett. 2016;21(4):11782-11790.

114. Miladi H, Zmantar T, Chaabouni Y, et al. Antibacterial and efflux pump inhibitors of thymol and carvacrol against foodborne pathogens. Microb Pathog. 2016;99:95-100. doi:10.1016/j.micpath.2016.08.008

115. de Morais Oliveira-Tantino CD, Tantino SR, Limaverde PW, et al. Inhibition of the essential oil from Chenopodium ambrosioides L. and α-terpinene on the NorA efflux-pump of Staphylococcus aureus. Food Chem. 2018;262:72-77. doi:10.1016/j.foodchem.2018.04.040

116. Limaverde PW, Campina FF, da Cunha FAB, Crispim FD, Figueredo FG, Lima LF. Datiane de M Oliveira-Tinto C, de Matos YMLS, Morais-Braga MFB, Menezes IRA, Balbino VQ, Coutinho HDM, Siqueira-Júnior JP, Almeida JRGŠ, Tantino SR. Inhibition of the Tet(K) efflux-pump by the essential oil of Chenopodium ambrosioides L. and z-terpinene on the Staphylococcus aureus IS-58. Food Chem Toxicol. 2017;109(Pt 2):957-961.

117. Chovanová R, Mezovská J, Mikulášová M. The inhibition of the Tet(K) efflux-pump by the essential oil of Staphylococcus epidermidis by essential oils from three Salvia species. Lett Appl Microbiol. 2015;61(1):58-62.

118. Bukvić D, Ciric A, Soković M, et al. Micromeria thymifolia essential oil suppresses quorum-sensing signaling in Pseudomonas aeruginosa. Nat Prod Commun. 2016;11(12):1903-1906. doi:10.1177/1934578X1601101232

119. Sharifi A, Ahmadi A, Mohammadzadeh A. Streptococcus pneumoniae quorum sensing and biofilm formation are affected by Thymus
daenensis, Satureja hortensis, and Origanum vulgare essential oils. *Acta Microbiol Immunol Hung*. 2018;65(3):345-359. doi:10.1556/030.65.2018.013

120. Snoussi M, Noumi E, Punchappady-Devasya R, et al. Antioxidant properties and anti-quorum sensing potential of *Carum* *opticum* essential oil and phenolics against *Chromobacterium violaceum*. *J Food Sci Technol*. 2018;55(8):2824-2832. doi:10.1007/s13197-018-3219-6

121. Khan MSA, Zahir M, Hasan S, Husain FM, Ahmad I. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett Appl Microbiol*. 2009;49(3):354-360. doi:10.1111/j.1472-765X.2009.02666.x

122. Olivero-Verbel J, Barreto- Maya A, Bertel-Sevila A, Stashenko EE. Composition, anti-quorum sensing and antimicrobial activity of essential oils from *Lippia alba*. *Braz J Microbiol*. 2014;45(3):759-767. doi:10.1590/S1517-83822014000300001

123. Bjarnsholt T. The role of bacterial biofilms in chronic infections. *APMIS Suppl*. 2013;121(136):1-58. doi:10.1111/apm.12099

124. Wang Y, Venter H, Ma S. Efflux pump inhibitors: a novel approach to combat efflux-mediated drug resistance in bacteria. *Curr Drug Targets*. 2016;17(6):702-719. doi:10.2174/1389450116666151001103948

125. Defoirdt T. Quorum-sensing systems as targets for antivirulence therapy. *Trends Microbiol*. 2018;26(4):313-328. doi:10.1016/j.tim.2017.10.005

126. World Health Organization. Tuberculosis. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

127. Kazemian H, Heidari H, Yamchi JK, et al. In vitro anti-mycobacterial activity of three medicinal plants of Lamiaceae family. *Reant Pat Antiinfect Drug Discov*. 2019;13(3):240-245. doi:10.2174/1574891X13666180626170155

128. Zanetti S, Cannas S, Molicotti P, et al. Evaluation of the antimicrobial properties of the essential oil of *Myrtus communis* L. against clinical strains of Mycobacterium spp. *Interdiscip Perspect Infect Dis*. 2010;2010 pii:931530. doi:10.1155/2010/931530

129. Sieniawska E, Sawicki R, Golus J, et al. *Nigella damascena* L. essential Oil—A valuable source of β-Elemene for antimicrobial testing. *Molecules*. 2018;23(2):256. doi:10.3390/molecules23020256

130. CBS News. Could antibiotic-resistant “superbugs” become a bigger killer than cancer? Holly williams. Updated April 21, 2019. https://www.cbsnews.com/news/could-antibiotic-resistant-superbugs-become-a-bigger-killer-than-cancer-60-minutes-2019-04-21/

131. Chang Q, Wang W, Regev-Yochay G, Lipsitch M, Hanage WP. Antibiotics in agriculture and the risk to human health: how worried should we be? *Evol Appl*. 2015;8(3):240-247.

132. Tang KL, Caffrey NP, Nobrega DB, et al. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet Plan Health*. 2017;1(8):e316-e327.

133. Owen L, Laird K. Synchronous application of antibiotics and essential oils: dual mechanisms of action as a potential solution to antibiotic resistance. *Crit Rev Microbiol*. 2018;44(4):414-435. doi:10.1080/1040841X.2018.1423616

134. Lahmar A, Bedoui A, Mokdad-Bzeouich I, et al. Reversal of resistance in bacteria underlies synergistic effect of essential oils with conventional antibiotics. *Microb Pathog*. 2017;106:50-59.

135. Langeveld WT, Veldhuizen EJA, Burt SA. Synergy between essential oil components and antibiotics: a review. *Crit Rev Microbiol*. 2014;40(1):76-94. doi:10.3109/1040841X.2013.763219