

## LACTOBACILLI AND CLOVE ESSENTIAL OIL AGAINST MENACE OF E.COLI: A POSSIBLE SOLUTION FOR ANTIBIOTIC RESISTANCE

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### ABSTRACT

*E.coli is one of the most common diarrheagenic gram negative bacteria also known to cause urinary tract infections. Treatment of its infection has become more problematic due to development and dissemination of its antibiotic resistant pathotypes. This review focuses on lactobacilli and clove essential oil as alternatives that have been found effective in treatment of E.coli with their reported modes and degree of action. A combined treatment of both lactobacilli and clove essential oil under optimized conditions for their highest possible activities in presence of each other can be a new regime to decrease dependency on conventional antibiotics and thus, control rising antimicrobial resistance.*

**KEYWORDS:** Anti microbial resistance, Lactobacilli, Clove essential oil.

### INTRODUCTION

**Background:** The well-known gram negative coliform bacteria, *Escherichia coli* (*E.coli*) is responsible for a number of infectious diseases like urinary tract infections, gastroenteritis, colitis, diarrhea etc. Its diverse pathotypes have been recognized as major public health concern. Infectious diarrhea due to pathogenic bacteria in particular, is a major health problem in India and other developing countries. There are a number of significant reports showing many diarrheagenic *E.coli* pathotypes round the globe.<sup>[1]</sup> Enterotoxigenic *E.coli* is also among the most frequent causative agents of traveller's diarrhea.<sup>[2]</sup> Deaths of children of age less than 5 years due to diarrhea was approximated to be 1.87 million at global level

(with an uncertainty range of 1.56 to 2.19 million), which is nearly 19% of total child deaths.<sup>[3]</sup> Taking in to account the pathogenesis of diarrhea by *E.coli*, it has been known that the entero-hemorrhagic and entero- pathogenic *E.coli* destroys the microvilli of the intestine to form attaching and effacing (A/E) lesions on infected cells leading to diarrhea.<sup>[4]</sup>

Another common community acquired and hospital acquired infection is the urinary tract infection (UTI). UTI exerts its impact in the form of clinical syndrome over millions of patients in the world effecting mostly women who are healthy otherwise.<sup>[5]</sup> UTI occurs mainly due to invasion of the bladder, urethra or kidneys by pathogens.<sup>[6]</sup> Uropathogenic *E. coli* is responsible for nearly 90% of all urinary tract infections.<sup>[7]</sup> Urinary tract infections are most prevalent in India and the world and the most common bacteria present in such cases is *E.coli*.<sup>[48]</sup> Worldwide spread of different clonal groups of *E. coli* was one of the major issues in the epidemiology of urinary tract infections.<sup>[8]</sup>

Uropathogenic *E. coli* is known to colonize the urinary tract and it may also ascend to the ureters and establish a secondary infection, acute pyelonephritis with irreversible kidney damage. It causes nosocomial and community acquired infections, both in adults and children.<sup>[9]</sup> Most of the studies conducted in recent times report *E.coli* to be the commonest pathogen isolated from UTI patients.<sup>[10,11]</sup> According to Tan and Chlebicki (2016), *E.coli* is the predominant uro- pathogen in acute community-acquired uncomplicated UTI.<sup>[10]</sup> A study done by Ahmed et al (2015) among cancer patients in North India reported *E.coli* to be predominant pathogen both in community acquired UTI (68%) and hospital acquired UTI (45%) followed by *Klebsiella spp* and *Enterococcus spp*.<sup>[12]</sup> Another study done in medicine department at a tertiary hospital in Bangalore reported that gram negative bacteria constitute the largest group causing UTI (prevalence = 84.1%) with *E.coli* being the commonest (70%) uro-pathogen.<sup>[13]</sup>

**Problems associated with available treatment regime for *E.coli* infection:** Apart from maintenance of adequate rehydration as primary treatment regime in case of *E.coli* diarrhea, a number of other treatment options are available for diverse range of pathogenic *E.coli* like antibiotic administration in case of extra-intestinal infections.<sup>[14]</sup>  $\beta$ -lactam antibiotics have been successfully used in such cases<sup>[15, 16]</sup>, but indiscriminate use of antibiotics has now led to emergence of antibiotic resistant *E.coli*. The emergence and dissemination of antibiotic resistant bacteria is a very important public health issue because such organisms when cause infections are difficult to treat. Emergence of anti-microbial resistance in uro-pathogenic

bacteria has further complicated the therapeutic decisions. Resistance to the antibiotic ciprofloxacin is increasing worldwide, that too especially in bacteria which cause UTIs.<sup>[17]</sup> Empirical therapy failures are also common due to emergence of extended spectrum  $\beta$ -lactamases.<sup>[6]</sup> High resistance to cotrimoxazole,  $\beta$ -lactam antibiotics, fluoroquinolones and 3<sup>rd</sup> generation cephalosporins has also been noted in some studies.<sup>[12,13]</sup> Most of these bacteria have now developed resistance to the  $\beta$ -lactam drugs by producing Extended Spectrum  $\beta$ -Lactamase (ESBL) enzymes.<sup>[18]</sup> There has been an increase in the resistance towards fluoroquinolones, trimethoprim and cephalosporins among urinary tract infection cases in recent years, which is quite worrisome. There is increased prevalence of *E. coli* strains producing extended spectrum  $\beta$ -lactamase, Amp C and Metallo- $\beta$ -lactamase, making the clinical management even more difficult in hospitalized patients.<sup>[19,20]</sup> Therapeutic options for ESBL producing *E. coli* is treatment with Nitrofurantoin, Fluoroquinolones (Norfloxacin, Ofloxacin, Ciprofloxacin etc), Fosfomycin, Carbapenems (Meropenem, Imipenem), Mecillinam or Tigecyclin.<sup>[18]</sup> Production of ESBL enzymes in *E. coli* may be a risk factor for development of resistance towards Carbapenemase in near future.<sup>[18]</sup> A recent study done by Sekar et al in 2016 regarding prevalence of *E. coli* and *Klebsiella* in rural South India shows that prevalence of aztreonam and fluoroquinolone resistance was very high in *E. coli*.<sup>[21]</sup> Another study done by Maheshwari et al (2016) revealed a potential health threat due to common presence of ciprofloxacin resistant ESBL producing enteric bacteria (mainly *E. coli*) in hospital waste water and clinical sources.<sup>[22]</sup> Not only in India, but in whole Asia-Pacific region, the antibiotic resistance is a serious problem.<sup>[4]</sup> Antibiotic therapies are therefore posing a risk of transforming bacteria to a multidrug resistant strain.

With passage of time, *E. coli* has evolved in a number of ways in order to survive and continues to cause community acquired and nosocomial infections. Infection outbreaks occur due to emergence of newer and more virulent strains. *E. coli* causes intestinal and extra-intestinal diseases by acquiring virulence factors through genetic recombination, horizontal gene transfer and natural selection.<sup>[23,24]</sup> For eg. O104:H4 is a new strain of *E. coli* isolated from patients of bloody diarrhea and hemolytic uremic syndrome, which caused an outbreak in Germany.<sup>[25]</sup> The new strains also exhibit resistance to many drugs for which previous forms of *E. coli* were sensitive. For eg. the O104:H4 strain of *E. coli* causes serious complications during infections that ultimately lead to death, due to its resistance to many drugs.<sup>[25]</sup> The highly virulent O104:H4 strain of *E. coli* is multi-drug resistant due to horizontal gene transfer. It has multi-drug resistance protein encoding genes and also a

number of virulence proteins like Shiga toxin, which it gained through prophage elements acquired from its micro-environment.<sup>[25]</sup> Another way of development of resistance toward drugs is through amino acid substitutions that cause structural alterations which reduce the affinity of the drug for the receptor. For eg. resistance in *E.coli* towards quinolones (used in treatment of traveller's diarrhea) is caused by substitutions in the QRDR region of gyrA subunit of DNA gyrase.<sup>[2]</sup> Resistance of the Enterobacteriaceae to antibiotics, especially of the  $\beta$  lactam type has risen due to the mobilization of continuously expressed single genes encoding efficient drug modifying enzymes. Ubiquitous and strong selection pressure is accompanied by a shift from naturally occurring resistance like membrane impermeability, drug efflux etc to the new set of mobile gene pools which determine the epidemiology of modern antibiotic resistance. Antibiotic resistance is more available now to organisms like *E.coli*.<sup>[26]</sup>

The problems further worsen due to wrong management of the infection and/or over treatment.<sup>[13]</sup> Another revealing study showed 96% (4% gram positives) of total isolated microbes from UTI cases to be gram negative bacteria, among which *E.coli* was most prevalent. Upon antibiotic sensitivity testing of the isolates by disc-diffusion method with thirteen mechanistically different antibiotics, the gram negatives showed more resistance towards these antibiotics as compared to gram positive bacteria.<sup>[11]</sup> Nearly 1/4<sup>th</sup> of the UTI cases show recurrences in another study.<sup>[5]</sup> One more study done for evaluation of risk factors for and molecular characteristics of community-onset extended-spectrum cephalosporin-resistant (ESC- Enterobacteriaceae (EB) urinary tract infections (UTIs) in a US health system, showed that use of trimethoprim-sulfamethoxazole, diabetes, older age etc were associated with community- onset ESC-R EB UTI.<sup>[27]</sup> Thus, this problem of antibiotic resistance among the members of enterobacteriaceae including *E.coli* is prevalent in many developing and developed countries.

**Probiotics (*Lactobacilli*) against pathogenic *E.coli*:** Due to rising risk and side effects of using antibiotics, the need for alternative therapies which can either completely replace antibiotics or at least decrease dependency on them for treatment of diseases, has been increasingly felt. Probiotics, known to improve digestion, immunity, general health and well being have been under consideration in this regard since a few years. Probiotics have multiple modes of action, such as preventing pathogenic bacterial growth, binding to or penetrating pathogens, mucosal barrier function stimulation or changing immuno-regulation. With

growing problems associated with antibiotics utilization, the relevance of researching bacteria like *lactobacilli* for their probiotic function has gained momentum. Many recent studies involve the use of probiotics as alternative therapeutics against infectious agents. Vergis et al (2016) evaluated the *in vitro* and *in vivo* antimicrobial effects of probiotics (*lactobacilli*) and found them to be quite effective.<sup>[28]</sup> Earlier, many probiotic species were known to block epithelial adhesion and invasion by microbial pathogens *in vitro*, but their proven utility in management of clinical infections is limited to increasing recovery from acute infectious diarrhea and prevention of antibiotic-associated diarrhea.<sup>[29]</sup>

Since a long time, interest in use of probiotics as a safe way of changing the intestinal bacterial flora has increased. Probiotics have potential in many gastroenterological conditions, especially when the intestinal flora is disturbed.<sup>[30]</sup> Many *lactobacilli* species have long been used as probiotics and the antagonistic activity of some *lactobacilli* was known to be quite pronounced towards a few pathogenic and opportunistic bacteria like *shigellae*, enteropathogenic *Escherichia*, *proteus*, *staphylococci*.<sup>[31]</sup> *Lactobacilli* produce many antimicrobial substances such as hydrogen peroxide, organic acids and bacteriocins.<sup>[32]</sup> Not only this, *lactobacilli* are able to compete with, exclude and displace pathogenic gastrointestinal bacteria however the degree of inhibition of adhesion depended upon the type of bacterial strain.<sup>[33]</sup> Scientific evidence is available proving successful treatment of diarrhea patients with *Lactobacillus GG*, *Lactobacillus reuteri*.<sup>[30]</sup> Enteral administration of *Lactobacillus R2LC* has been reported to attenuate endotoxemia and bacteremia associated with intra-abdominal infection in rats.<sup>[34]</sup> Taking in to consideration the effect of *lactobacilli* on uropathogens, it has been found that these benefiting bacteria can be used to coat surfaces of biomaterials to decrease the adhesion of uropathogens over them.<sup>[35]</sup>

*Lactobacilli* have also been used in combination with other antimicrobial agents. For eg. a study of antimicrobial activity of *lactobacilli* against uropathogens showed that *lactobacilli* and low dose ampicillin have a positive effect on treatment of *E.coli* in a UTI model.<sup>[36]</sup> There have also been evidences which support the notion that *lactobacilli* in combination with antimicrobial agents are effective against pathogenic biofilms.<sup>[37, 38]</sup> Taking in to account *E.coli* particularly, it has been reported that *lactobacilli* have ability to coaggregate with it, and this property of a specific *lactobacillus* species can play a role in identification of a potential probiotic that can be therapeutically used against pathogens causing urogenital infections.<sup>[30]</sup> In addition to restoring healthy urogenital microbiota, *lactobacilli* may displace

uropathogenic *E.coli* through acidification of the environment and reduce infection.<sup>[40]</sup> Thus, *lactobacilli* are promising alternative therapeutic agents against a number of gastrointestinal pathogens and uropathogens including *E.coli*.

***Clove essential oil as a potent anti-E.coli agent:*** For centuries, plant essential oils have been used empirically for treatment of infections but their scientific studies gained momentum recently when the need for alternative therapies was realized. A large volume of research has given strength to use of these natural compounds in a variety of fields like dentistry, food industry etc. Clove (*Eugenia caryophyllata*) essential oil, well known for its antibacterial and antifungal properties was found to be one of the highest active agents against 25 different genera of bacteria.<sup>[41]</sup> A study done by L. Nuñez and M. D' Aquino (2012) to test the activity of clove essential oil against *S.aureus*, *P.aeruginosa* and *E.coli* showed that *E.coli* was most sensitive to the clove oil amongst the three pathogenic bacteria.<sup>[42]</sup> The presence of eugenol makes clove oil a very potent antibacterial agent. Eugenol has ability to denature proteins in addition to reacting with cell membrane phospholipids leading to change in their permeability, thus inhibiting many gram positive and gram negative bacteria, yeast etc.<sup>[43,44]</sup> Eugenol is also reported to inhibit quorum sensing at sub-inhibitory concentrations.<sup>[45]</sup>

A large number of research studies involving the determination and comparison of antimicrobial activities of different essential oils have demonstrated fairly good antimicrobial activity of clove essential oil against many groups of pathogens including multi-drug resistant bacteria thus, reinforcing its therapeutic utility.<sup>[46, 47, 48]</sup> Thus, clove essential oil is a highly eligible candidate to be considered for use alone or in combination with other anti-microbial compounds for treatment of *E.coli* infections, whether it is diarrhea or urinary tract infection, thereby reducing the dependency on antibiotics.

***Application of combined treatment of clove essential oil and Lactobacilli:*** Studies report that the minimum inhibitory concentrations (MICs) of some essential oils against pathogenic bacteria are lower as compared to those for probiotic bacteria.<sup>[49]</sup> So, a well optimized strategy of developing a combined effect of essential oil and probiotics where the essential oil's dose is sufficient enough to wipe out the pathogenic bacteria without harming the probiotic bacteria can be very effective. Only a sublethal effect of clove essential oil was observed against probiotic bacterial species *L. rhamnosus*, indicating the resistance of these microorganisms to clove essential oil.<sup>[50]</sup> This hints towards the possibility of harnessing their combined effect against pathogenic microbes. On the basis of many evidences of high



antimicrobial activity of both *Lactobacilli* and clove essential oil against *E.coli*, the chances of their successful combinational use against this pathogen to act in a synergistic way, are fairly high. Minimum inhibitory concentrations of clove essential oil against *lactobacilli spp.* and *E.coli*, the optimum conditions for best activities of both-clove essential oil and *lactobacilli*, are a few criteria required to be considered to gain an overall increase in their combined anti-microbial actions.

## CONCLUSION

Clove essential oil and *Lactobacilli* both have proven high anti-microbial potential. In order to harness their microbicidal activity, rigorous research involving combinational studies and optimization of conditions to utilize their combined potential are required. This can be a new breakthrough in tackling the widespread rise of antimicrobial resistance.

## Declarations

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