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REVIEW ARTICLE

Tea: An anti-bacterial agent against drug resistant bacteria

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ABSTRACT

Antibiotics have played a crucial role in the treatment of bacterial infections. Past few decades are marked with advancement of multidrug resistant (MDR) pathogens, which have endangered antibiotic's therapeutic efficacy. Scientific world is now struggling with the crisis of MDR pathogens. This supreme matter demands careful attention or otherwise it would jeopardize clinical management of infectious diseases. Implication of alternative approaches can pave a new way in the treatment of these troublesome bacteria. Tea leaves are known to pose antibacterial activity against many pathogenic microorganisms. This review has summarized the antibacterial potential of tea leave's extracts against resistant bacterial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Acenitobacter spp*, *Campylobacter spp.* Consumption of natural products such as tea may very well replace, minimize or obliterate this complicated situation.

Keywords: Anti-bacterial**,** Tea**,** *Camellia sinensis***,** Drug resistant bacteria, Antibiotic resistant bacteria, Synergism, Polyphenols.

INTRODUCTION

Infectious diseases represent the 3rd major cause of death globally (Farooqui *et al*., 2015). Emergence of infectious diseases due to drug resistant bacteria has resulted in serious health threats (Radji *et al*., 2013). Antimicrobial resistance (AMR) has been declared as one of the biggest health threat humanity is facing (World Health Organization, 2020). Poor health care facilities and the misuse of antibiotics have further escalated this situation (Aliasghari *et al*., 2017).

Drug resistance is a burning issue of the 21st century. The encountering side effects are always linked with the adverse use of synthetic drugs (Anita *et al*., 2015). Emergence of drug resistance among bacterial species is a global call to seek an alternative way to treat worrisome resistant bacterial species responsible for infectious diseases. This alarming situation can be overcome with the discovery of new antibiotics or highlighting the possible alternatives to curb infectious disease. As bacteria evolve resistance mechanism against first line of antibiotics, second or third group of antibiotics are used – associated with adverse side effects, more expensive as compared to other groups (Radji *et al*., 2013).

Due to the time to time changes in the disease pattern of the infectious diseases and resistant microbial strains emerging to current antibiotics, our need to treat microbial infections effectively requires fresh approaches (Taylor *et al.*, 2002). Botanicals can serve as resistance modifiers to combat infectious microorganisms (Gupta & Birdi, 2017). The development of plant-derived antimicrobials has been greatly focused upon, especially the polyphenols derived from plants (Friedman, 2007). Most polyphenolic compounds in natural food sources have been found to benefit humans by displaying different antibacterial, antiviral, anticancer and anti-inflammatory activities (Scalbert *et al.,* 2005). Polyphenols are abundantly found as major constituents of tea leaves (Almajano *et al.,* 2008).

Tea is second to water as being the most widely used non-alcoholic, cheapest beverage. Mostly Asian countries i.e. People's Republic of China, India and Sri Lanka are the major producers of tea (FAOSTAT, 2018). Green, oolong, pu-erh and black tea are the forms in which tea is generally consumed (Balentine *et al.,* 1997). Tea is cultivated as monocrop *Camellia sinensis* which belongs to Theaceae family. *Camellia sinensis* is effective against drug resistant microorganisms (Anita *et al*., 2015). It is beneficial against a variety of bacterial population residing in our community having pathogenic or opportunistic nature. Tea is an important medicinal beverage with diverse antimicrobial properties (Reygaert, 2014).

This review mainly focuses on the antibacterial activity of *Camellia sinensis* on different drug resistant bacteria. It also discusses the synergistic effects of tea with some antibiotics. The main goal is to investigate and identify the antibacterial capabilities of *Camellia sinensis*, so that its application in the management of infectious diseases can be taken into account.

Tea types and manufacturing process.

To manufacture tea, two major varieties of *Camellia sinensis* are used which include *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* (Lin *et al.,* 1998). On the basis of manufacturing process there are different types of tea: green tea is the non-fermented form (produced when the fresh leaves are dried and steamed to inactivate the polyphenol oxidase thus, preventing oxidation); oolong tea is the semi-fermented form (produced when the fresh leaves are partially fermented then dried); white tea is non fermented form; black and pu-erh tea is fermented form, post-harvest fermentation is followed by steaming and drying. Polyphenol oxidase catalyzes oxidation and fermentation of black tea, while pu-erh tea is produced by microbial fermentation (Bancirova, 2010).

Chemical composition of tea: The composition of tea can vary due to changes regarding the climate, plant varieties, leaf age, and season. Tea is chemically composed of amino acids, alkaloids, carbohydrates, volatile compounds, proteins, polyphenols and certain trace elements. Polyphenols are of great importance due to their biological activity (Cabrera *et al.,* 2003).

Among the polyphenols, particularly flavonoids are important commonly flavan-3-ols. As compared to other foods, concentration of flavan-3-ols is higher in tea. On the basis of polymerization, subclasses of flavan-3-ols have been recognized. The monomers include catechins such as catechin (C), gallocatechin (GC), epicatechin (EC), epigallocatechin (EGC), Epicatechin gallate (ECG) and epigallocatechin-3-gallate (EGCG). The dimers include theaflavins such as theaflavin-3, 3′-digallate (TF3), theaflavin-3′-gallate, theaflavin-3-gallate and theaflavin. The oligomers include derived tannins. Flavones and flavonols are other flavonoids present in lesser amounts (Peterson *et al*., 2005). The most abundant and biologically active catechin is the EGCG (Bansal et *al.*, 2012).

Antibacterial activity of tea polyphenols against drug resistant bacteria: The most potent catechins showing antibacterial activity are EGCG and ECG (Hamilton- Miller, 1995). The mechanism of antibacterial activity of tea catechins is explained by a few hypotheses. The biosynthesis of peptidoglycan is interfered by EGCG. EGCG induces precipitation by direct binding with

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peptidoglycan, acts as major mechanism against *Staphylococcus* (Shimamura *et al.,* 2007). Another study suggested that EGCG, in the presence of superoxide dismutase, reacted with reactive oxygen species, generating hydrogen peroxide, thus showing bactericidal activity (Arakawa *et al.,* 2004). Antibacterial activity of different types of tea polyphenols are evaluated against different types of bacteria such as *Helicobacter, Bacillus, Clostridium, Streptococcus, Staphylococcus aureus and Pseudomonas aeruginosa* (Bansal *et al.,* 2013).

Helicobacter pylori: Helicobacter pylori are a chief causative agent of most gastroduodenal diseases, which are considered to be infectious diseases, required to be treated with antibiotics (Gut, 2004). Interesting but frightening piece of information is the resistance pattern in H.pylori that exhibits regional differences (Wang et al., 2019). H. Pylori strains have crossed the threshold level and now have reached alarming levels of antimicrobial resistance (Savoldi et al., 2018). Treatment of choice in H.pylori infections is triple therapy comprising of clarithromycin, amoxicillin and a proton pump inhibitor (PPI). Second alternative is the use of sequential drug treatment: primary treatment with PPI and amoxicillin followed by clarithromycin and metronidazole. However, the treatment may fail as a result of certain reasons; most important one is the resistance development against clarithromycin (Mégraud F, 2003). In vitro efficiency of tea catechins was investigated against H. pylori strains, highly resistant to metronidazole (MTZ) and clarithromycin (CLR) (Yanagawa et al., 2003). Only two tea polyphenols (EGCG and ECG) were found to be effective against H.pylori with MIC_{90} value 100µg/ml. The general combination effect between EGCG, CLR, and MTZ was determined by measuring fractional inhibitory concentration (FIC) using checkerboard assay. Presence of only 50ug/ml of EGCG decreased MIC of MTZ and CLR from 100µg/ml to 6.25 µg/ml (FIC 0.56) and 100 µg/ml to 1.25 µg/ml (FIC 0.75), respectively. Potent anti-H.pylori action of tea catechins is inhibition of urease enzyme (Baker, 2020).

Acinetobacter baumanii: Plasticity in the genome of Acinetobacter baumanii has enabled this bacterium to develop resistance against a number of antibiotics including carbapenems (Shin & Woojun, 2017) and colistin (Li & Rayner, 2006): which are considered to be the last resort for treating A. baumanii infections. Emergence of A.baumaniii as formidable resistant pathogen is a challenge. A.baumanii once termed as "Iraqibacteria" (Aoife et al., 2012) was suspected to be transmitted through the soil of Iraq and Afghanistan. Clinical inspection of soil, however, paved the way to understand the cause and transmission of infections. A. baumanii complex comprising A.baumanii, A. pitti and A.nosocomials is responsible for 95% of all infections caused by this class of gammaproteobacteria (Nemec et al., 2011). A.baumanii is a chief causative agent of ventilator associated pneumonia. Not only restricted to nosocomial infections (Oncul et al., 2002), this bacterium causes number of infections: soft tissue infections, urinary tract infections and secondary meningitis. Horizontal gene transfer (Poirel et al., 2011) and genomic structure confers resistance issues in the A. baumanii infections treatment. Antibiotic efflux, enzymatic and non-enzymatic degradation of antimicrobial drug, alteration in antibiotic binding site are evident as resistance mechanisms in A.baumanii (Shin & Woojun, 2017). Monotherapy to eliminate A.baumanii infections has now been replaced by drug's regiment.

An investigation was designed to monitor the efficacy of water soluble extract of green tea against Acinetobacter sp, isolated from burn wounds of patients, hospitalized in Tehran, Iran. Among 20 bacterial strains, 75% were reported to develop resistance against 12 antibiotics tested.Antimicrobial resistance profile was developed by taking into account all major classes of antibiotics such as penicillins, tertracyclines, cephalosporins, aminoglycosides and carbapenems. Minimum bactericidal concentration of water soluble green tea extract was determined by Muller-Hinton agar culturing. The average MBC of green tea aqueous extract against all the selected isolates was found to be 387.5±127.6µg mL-1 (Jazani et al., 2007).

Tea polyphenols have been tested against *A.baumanii* strains to estimate the effectiveness of tea components alone or in combination with other antimicrobials. Minimal inhibitory concentration required to kill *A. baumanii* was measured. Green tea component epigallocatechin gallate (EGCG) was found to kill *A.baumanii* strains (Osterburg *et al.,* 2009). Dosage of 2.5mg/ml was found to produce largest clearing zone. Summarization of the results indicated a decrease in the clearing zone with a decrease in dose of polyphenol used. Checkerboard assay to evaluate the synergistic effect of EGCG and mafenide-acetate (5%) as topical agent was performed. Three highly resistant strains and one susceptible strain were subjected to susceptibility testing to evaluate fractional sub-inhibitory concentration. For further studies, four isolates were selected (one-susceptible and three highly resistant *A.baumanii* strains). One of the resistant isolate demonstrated synergy between EGCG and mafenide acetate at concentrations of 0.039µg/µL. and 0.625% respectively, while for others, partial synergy was observed. To evaluate EGCG antibacterial efficacy, time kill assay was performed on two highly resistant isolates and within first 5 hours of incubation 3-log reduction in CFU/ml was reported. Microscopic analysis showed destruction of cells following 5 hours of exposure to EGCG (Osterburg *et al*., 2009).

In an investigation, synergistic effect of EGCG and curcumin (extracted from turmeric plant *Curcuma longa*) was evaluated on *A.baumanii* susceptible type strain ATCC 19606 and eight multi-drug resistant strains. MDR strains were found to be neither inhibited nor killed by colistin, tigecycline and β-lactams. MIC for EGCG ranged between 128-1024µg/ml. Synergistic effect of curcumin (CCM) and EGCG was studied using checkerboard assay. Synergistic effect appeared against 5 out of 9 isolates and remaining four strains exhibited additive effect. Presence of EGCG reduced CCM minimum inhibitory concentration by 3-7 folds with the greatest interaction achieved at CCM concentration of 4µg/ml. The possible mechanism of synergistic effect is the H_2O_2 production by EGCG, which drives destruction of outer membrane and allows entry of CCM in *A.baumanii* cells (Betts *et al.,* 2014).

Table I: A comparative review of different studies illustrating effect of tea extracts on *A.baumanii* strains and methodologies adopted

Methods	Findings	References
Muller Hinton agar assay	7mm diameter observed	Osterburg *et al*., 2008
*Minimal inhibitory concentration	MIC_{50} =0.312mg/ml MIC_{90}=0.625mg/ml	
Time kill assay	3-log reduction in cfu/ml	
Checker-board assay	EGCG =0.03µg/ml Sulfamylon=0.625%	
MIC	128-102 µg/ml	Betts *et al*., 2014
[1]Time kill assay CCM 1:8 EGCG 1:4	4-5 log reduction	
[2]Checker board assay	3 to 7 log reduction in MIC of CCM	
MIC_{90}	256 µg/ml	Betts *et al*., 2014
MBC_{86}	512 µg/ml	
Fractional inhibitory concentration	> 0.5	
Time kill assay	>2 log 10	
**Minimum bactericidal concentration	387.5±127.6 µg mL-1	Jazani *et al.,* 2007
Disc diffusion method	Synergism was reported between tea Epicatechin and Theaflavin	Betts. J., 2011

*Lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism afterovernight incubation (Andrews, 2001)

**The concentration where 99.9% or more of the initial inoculum is killed' or 'the lowest concentration at which no growth is observed after sub-culturing into fresh broth' (Tajkarimi, Ibrahim, & Cliver, 2010)

[1]The time-kill test reveals a time-dependent or a concentration-dependent antimicrobial effect (Pfaller *et al.,*2004)

[2]A method to evaluate interactions between biologically active agents such as synergism, zero interaction, andantagonism (Martinez-Irujo *et al*. 1996)

Minimal inhibitory concentration and minimal bactericidal concentration (MBC) of EGCG on 63 resistant *A.baumanii* isolates were clinically tested: all strains were resistant to β-lactam drugs,5

strains developed resistance against aztreonam, carbenicillin and ceftazimide, in remaining two strains, and resistance was reported against all classes of drugs including polymixins. MIC of EGCG was calculated using a concentration range of 64-1024µg/ml. Minimal bactericidal concentration was measured by growing cells on LB agar plates containing EGCG in concentration range used for MIC measurements. Time kill assay was used for analyzing tea's component effectiveness. Checkerboard assay was used to evaluate synergistic or antagonistic relationship between tea EGCG and antibiotics. EGCG (128µg/ml) in combination with N-Methyl-2-pyrrolidone (NMP) efflux pump inhibitor (100µg/ml) significantly enhanced *A.baumanii* susceptibility to different antimicrobials. Possible reason for reversal of resistance was binding of EGCG with resistance-nodulation-cell division (RND) type efflux components (Lee *et al*., 2017).

Betts *et al.*2011 found out antibacterial activity of black tea theaflavin and epicatechin against four clinical isolates of *A.baumanii*. Epicatechin alone showed not sensitivity against selected bacterial isolates. On the other hand, theaflavin exerted strong anti-bacterial effect. Significant synergistic effect above 1mg concentration was observed when theaflavin and epicatechin combination (2:1) was used. Underline mechanism of synergism was not understood.

Campylobacter: Transmitted through fecal-oral route, responsible for zoonosis and a causative agent of gastroenteritis (Capriolo et al., 1996), Campylobacter species have been recognized as the most important foodborne pathogen. In recent years, various strains of campylobacter have been found to develop resistance against fluoroquinolones, macrolides and tetracyclines. Extrusion of antibiotics by efflux pumps is crucial modification in outbreak of resistant strains (De Rossi et al., 2006). A wide range of natural chemicals are available to be tested for antimicrobial potential, reduction in toxicity; acting as potent substance. Clarithromycin, dirithromycin and tylosin, azithromycin resistant strains were selected for antibiotic susceptibility trials to test the existence of co-resistance. Seventeen strains along with three reference strains were used: seven strains developed co-resistance against all the four antibiotics tested, three strains were found to be resistant against erythromycin and azithromycin, one strain developed resistance against dirythromycin and tylosin. Effectivity of EGCG against macrolide resistant isolates was observed by using Muller-Hinton broth. The presence of EGCG reduced MIC of clarithromycin, dirythromycin, azithromycin by 4-6 folds in 60% of isolates. In contrast, tylosin MIC was found to be decreased by 4-16 folds in only 45% of isolates. CmeABC efflux pump played important role in the development of resistance against macrolides (Kurincic et al., 2012).

Staphylococcus aureus: *Staphylococcus aureus* is a member of family Micrococcaceae, a gram positive bacterium, present in single or group like structures. *Staphylococcus aureus* is present as normal flora in human beings on skin and in upper respiratory tract (nasal passages). *Staphylococcus aureus* can cause serious health problems by becoming opportunistic pathogen. It can cause different infections like pneumonia, mastitis, impetigo, cellulitis, osteomyelitis, endocarditis and bacteremia. About 90-95% *Staphylococcus aureus* strains have become resistant to penicillin (Casal *et al*., 2005). Before 1950's, benzyl penicillin was administrated in staphylococcal infections. It was a beta lactam antibiotic. In late 1950s, *Staphylococcus aureus* becomes resistant to benzyl penicillin by producing beta lactamases that cleave the beta lactam ring. Then more struggles were made to find such penicillin derivatives that were resistant to beta lactamase. The goal was achieved in 1959 with the synthesis of methicillin which had phenol group of benzyl penicillin di-substituted with methoxy groups. Methoxy groups provided steric hindrance because they surrounded the amide bond. Soon after methicillin was used clinically, methicillin resistant *Staphylococcus aureus* (MRSA) strains were isolated (Stapleton & Peter, 2002). The problem of resistance of bacteria against synthetic drugs has increased. In the emerging resistance of chemical agents, plant derived antibacterial agents are a source of new therapeutics (Hemaiswarya *et al*., 2006).

Among botanicals, green tea extract was found to exhibit antibacterial activity against beta lactamase producing *Staphylococcus aureus* to some extent by inhibition of beta lactamase secretion (Yam *et al*., 1998). Further studies indicated potential of green tea extracts as drug resistance reversal tool in MRSA. Underlying mechanism was inhibition of MRSA by blocking synthesis of Penicillin binding protein (PBP2') (Miller & Shah, 1999). The tea component epigallocatechin gallate showed strong synergistic effect against bacteria (Hu *et al*., 2002). The EGCG along with beta lactam antibiotic interferes with structure of cell wall by direct binding to peptidoglycan (Zhao *et al*., 2001).

An investigation was designed to evaluate the synergistic effect of tea polyphenols (TPP) along with antibiotic was tested against 30 clinical isolates of MRSA. According to HPLC chromatographic analysis, tea polyphenols collectively called catechins include five main compounds: 2% epicatechin (EC), 24% epicatechin gallate (ECG), 1% epigallocatechin (EGC) and 50% epigallocatechin gallate (EGCG). Oxacillin was used along tea polyphenols (TPP). Agar dilution method was used for determination of MIC of oxacillin, tea polyphenols and combined oxacillin-TPP against *Staphylococcus aureus* isolates. Out of 30 isolates, 17 were methicillin susceptible while other 13 were MRSA. The range of MIC of oxacillin was 8-512 (µg/mL) while the MIC range of TPP was 50-180(µg/mL). The tea polyphenols and oxacillin dramatically decreases the MIC in combined form. TPP showed synergistic effect with oxacillin. The bactericidal effect was analyzed by time kill assay. When antibiotic was used alone, it showed delayed growth but growth reached comparable level after 24hrs. In presence of TPP, there was initial drop in CFU for the first 8 hours and followed by bacterial numbers reached control values at 24 hrs. The synergistic effect showed that almost all bacteria killed after 24 hours (Cho *et al*., 2008).

Another study was designed to assess killing activity of green tea extract against *Staphylococcus aureus* and MRSA by disc diffusion method using 1 µg oxacillin and 16 µg green tea extract (GTE). In case of MRSA, oxacillin showed no inhibition zone while GTE showed inhibition zone of 19.13mm (Radji *et al*., 2013).

Antibacterial activity of green tea was evaluated against the isolates of *Staphylococcus aureus.* In one study, 26 Isolates were collected from different clinical samples like burns, wounds, teeth, skin, nasal, ear discharge and sputum. The susceptibility of isolates was tested against different antibiotics (cephalixin, ceftaxime, gentamicin, erythromycin and ciprofloxacin) by using disc diffusion method. Those isolates that showed resistance to antibiotics were selected for sensitivity test against green tea extract. Out of 26 isolates, 15 isolates were multi drug resistant. Different concentrations of green tea extract were used. The antibacterial activity of green tea extract was measured by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC). The zone of inhibition ranged between 7.5-16mm and the average of MIC and MBC was 4 and 5 mg/L respectively. This study showed the green tea extract has antibacterial activity against the multi-drug resistant *Staphylococcus aureus* isolates (Alsayigh, 2016).

In comparatively recent studies, in vitro antibacterial activity of green tea extract against *Staphylococcus aureus* and MRSA was evaluated. Around 15 consecutive laboratory isolates of S. aureus (MSSA) and MRSA each, isolated between August to September 2017 were inoculated in media containing green tea extract [epigallocatechin gallate (EGCG)] in concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml. After overnight incubation the bacterial broth was subcultured using standard calibrated loop (0.01ml) onto Mueller Hinton agar. The lowest concentration of EGCG in which no growth occurred was considered as the minimum bactericidal concentration (MBC). EGCG was effective against clinical isolates of MSSA and MRSA with MBC of 5mg/ml except 2 MRSA isolates for which MBC was 10mg/ml (Sowjana *et al.,* 2020).

Table II: A comparison of multiple studies conducted to evaluate efficacy of tea components or extracts against drug resistant *Staphylococcus aureus*

Table II: A comparison of multiple studies conducted to evaluate efficacy of tea components or extracts against drug resistant Staphylococcus aureus

Methods	Results	Reference
Disc diffusion method Minimum Inhibitory concentration Minimum bactericidal concentration	Inhibition zone =7.5 -16 mm, the Avg of MIC=4 and Avg of MBC =5 mg/ml, this is slightly higher in compared with values as previously reported, this difference in values could be largely attributed to the extraction process.	**Alsaihy, 2016** Number of isolates=26
Disc diffusion method Minimum Inhibitory concentration	The activity of 16 µg green tea extract against laboratory strain S. aureus ATCC 25923 was comparable to that of commercially available oxacillin (1 µg).	***Radji et al*.,2013** Number of isolates=2
Minimum Inhibitory concentration	EGCG, the main constituent (*50%) in our TPP preparation, is likely to be most responsible for TPP's antibacterial activity against S. aureus.	***Cho et al*., 2008** Number of isolates=13

Pseudomonas aeruginosa: Pseudomonas aeruginosa is an opportunistic and leading nosocomial pathogen. According to National Nosocomial Infection Surveillance (NNIS) System, data summary report from January 1992

Pseudomonas aeruginosa: Pseudomonas aeruginosa is an opportunistic and leading nosocomial pathogen. According to National Nosocomial Infection Surveillance (NNIS) System, data summary report from January 1992 through June 2004 states that about 10% nosocomial infections are caused by Pseudomonas aeruginosa. Such infections are life threatening because of least susceptibility of Pseudomonas aeruginosa against antibiotics and high rate of emerging resistance. It was initially found in patients of cystic fibrosis where constant infection with Pseudomonas aeruginosa leads to the sequential appearance of resistance to diverse antibiotic agents. The enhanced level of drug resistance is a result of the de novo emergence of resistance in a particular organism after contact with antimicrobials along with cross contamination between patients (Aloush et al., 2006). Green tea extract is a natural source with significant antibacterial prop*erties. This speculation leaded to a variety of research to check out the antibacterial potential of green tea extracts against MDR- P. aeruginosa.*

In a study, the antibacterial activity of green tea was tested against *P. aeruginosa* and MDR -

***P. aeruginosa* using disc diffusion method:** The antibiotic used was gentamycin. The inhibition zone of Green tea extract and gentamycin against *P. aeruginosa* and MDR -*P. aeruginosa* was measured and MIC was determined. The results showed that green tea was effective against *P. aeruginosa* but slightly less active comparable to gentamycin. Although green tea extract showed good activity against MDR -*P. aeruginosa* that is resistant to different antibiotics (Radji *et al*., 2013). The tea polyphenols act on cell membrane of MDR -*P. aeruginosa* increases the permeability of cell membrane that causes the disruption of cell membrane and resulted in death of cell (Yi *et al*., 2010).

Salmonella typhi: Salmonella typhi, a gram negative bacterium and a causative agent of typhoid fever, represents a public health concern. Typhoid fever associated with poor hygiene and lack of clean drinking water is endemic to Southeast, Far East Asia, Central and South America, Africa and Indian-subcontinent (Verma et al., 2010). Highly resistance Salmonella typhi (XDR S. typhi) has become the pressing health issue in Pakistan (Saeed et al., 2019). XDR S. typhi have developed resistance against all classes of antibiotics but macrolides and carbapenems (CDC, 2019). An extensive study was conducted to evaluate the efficiency of green tea aqueous and methanol extract against a wide multidrug resistant gram-negative S. typhi (Farooqui et al., 2015). Minimum inhibitory concentration was determined by microdilution broth assay and agar dilution method. Extracts were tested within concentration range of 5000-50µg/ml. Camellia sinensis extract exhibited higher antibacterial activity against MDR Salmonella typhi strains. Both aqueous and methanol extracts produced 6mm zone of inhibition on agar well diffusion Muller-Hinton agar plates. Formation of small zone of inhibition was explained by taking into account the high molecular weight of bioactive compounds and their slow diffusion through solidified medium. MIC for aqueous extract and methanol extract was reported to be 1.56mg/ml, and 1.25mg/ml, respectively while MBC for aqueous and methanol extracts was 1.56mg/ml and 2.5mg/ml respectively. Multi drug resistant Salmonella typhi strains (n=16) were found to be resistant to streptomycin, ampicillin, co-trimoxazole, tetracycline, nalidixic acid and ciprofloxacin. In an investigation, antimicrobial effect of tea extracts (black, green, white, purple tea processed from Kenyan germplasm) was examined against methicillin and penicillin resistant clinical isolate of S.typhi. Results showed that majority of tea extracts inhibited clinical isolate of S.typhi. Black tea extracts were reported to inhibit the S.typhi significantly. Negligible inhibitory effect was obtained with black tea buds processed from Kenyan germplasm. In contrast, white tea extracts processed from clone AHP S15/10 and TRFK 301/5 were found to produce significant inhibitory effect than any other selected isolate (Koech et al., 2013).

Klebsiella pneumonia: Klebsiella genus is responsible for 1/3rd of all infections associated with gram negative bacteria (Venezia et al., 2017). An infectious agent of pneumonia, septicemia, urinary tract, soft tissues and predominantly nosocomial infections, this bacterium is second to E.coli to cause blood stream infections (Meatherall, 2009). Moreover, it may lead to high mortality, extended hospitalization and high treatment cost (Giske et al., 2008). Emergence of resistant strains of Klebsiella pneumoniae is an increasing threat to public health (Effah et al., 2020). The development of drug resistance in K. pneumonia is associated with production of extended spectrum β-lactamases (Tawfik et al., 2011). In a study, antibacterial potential of green and black tea extracts was examined on Klebsiella pneumoniae isolates. 60% of all K. pneumoniae isolates from urine samples were multi drug resistant as a result of penicillinase production. Selected isolates were reported to develop resistance against cephalosporins, cefuroxime, norfloxacin and nalidixic acid. Methanol extracts of green tea and black tea showed maximum antibacterial activity against targeted resistant K.pneumoniae isolates with MIC of 0.4mg/ml (Labar et al., 2016). In another investigation effect of ethanol extract of green tea leaves on metallo- β- lactamase producing K.pneumoniae isolates M5 and M6) was monitored. Minimum inhibitory concentration of green tea extract (GTE) was determined by broth dilution method and minimum bactericidal concentration was evaluated by agar dilution method. MIC and MBC for GTE was calculated to be 11-12mg/ml and 12-13mg/ml respectively. Synergistic effect of GTE with ampicillin was examined by agar dilution method. ½ MBC of GTE dropped MBC of ampicillin from 10mg/ml to 400-500ug/ml (Tariq et al., 2015). In another study, anti-bacterial effect of epigallocatechin gallate (EGCG) against imipenem resistant Klebsiella pneumonia (IRKP) was explored, alone or in combination with imipenem. Minimum inhibitory concentration of EGCG against 12 clinical IRKP isolates was reported in the range 300-650 ug/ml. MIC of imipenem was reduced by 4-64 fold when co-incubated with 0.25 × MIC level of EGCG. Enhanced bactericidal activity against 12 IRKP isolates was observed using EGCG in combination with imipenem using time-kill method. Furthermore, proteomic analysis was performed. 2D polyacrylamide gel electrophoresis exhibited up-regulation and down-regulation of 4 and 8 genes in IRKP isolates when exposed to 1 × MIC OF EGCG. Scanning electron microscopy (SEM) confirmed the presence of wrinkles on the cell surface. EGCG posed synergistic effect in combination with imipenem mediated by stimulation in gene expression (Cho et al., 2011).

CONCLUSION

In this review, our discussion revolved around various studies about the antibacterial profile of tea and its polyphenols against drug resistant human pathogens. These studies can be taken into account to explore tea polyphenols action against bacteria as well as their virulence factors. The literature reveals that the most potent polyphenol against bacteria was EGCG. In comparison to Gram-negative bacteria, Gram-positive bacteria appeared to be more susceptible towards EGCG. Also, in the literature there is evidence that the tea polyphenols act synergistically with a variety of conventional antibiotics against drug-resistant and multidrug-resistant bacteria. For the treatment of human infections, the relevancy of tea polyphenols, their bio-availability, toxicity and *in vivo* activity should be studied in the future, considering the findings in the literature.

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