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Antibacterial Effects of Water Soluble Green Tea Extracts on Multi-Antibiotic Resistant Isolates of *Acinetobacter* sp.

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Abstract: In this research we evaluated the antibacterial activity of water soluble green tea extracts on isolates of *Acinetobacter*. A total of 20 strains were collected from burn wounds at different hospitals in Tehran, Iran. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. Antibacterial activity of water soluble green tea extract was measured by Minimum Bactericidal Concentrations (MBCs). Seventy five percent of isolated strains showed resistance to at least 12 antibiotics or more and all the strains were Multi-drug Resistant (MDR) strains. The average MBCs of the extract against all strains of *Acinetobacter* were 387.5±127.6 μg mL^{-1}. Present study suggests that green tea has significant bactericidal action on multi-drug resistant strains of *Acinetobacter*.

Key words: Green tea, MBC, *Acinetobacter*

INTRODUCTION

Acinetobacter sp. are ubiquitous, small, aerobic, short, plump, Gram-negative rods that prefer moist environments (Gerner-Smidt, 1995). They are also nonfermentative, oxidase negative, non motile, catalase positive saprophytes that could be distinguished from other bacteria by their lack of pigmentation (Ingram and Shewan, 1960). *Acinetobacter* sp. are usually considered to be opportunistic pathogens. They cause a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection and meningitis, especially in immunocompromised patients (Bergogne-Bérézin and Towner, 1996a) and patients admitted in intensive care and burn units (Humphreys and Towner, 1997), where they are frequent causes of ventilator-associated pneumonia and of bacteraemias (Bergogne-Berezin and Towner, 1996b). They are often multi-resistant to antibiotics, meaning that therapy and infection control are complicated (Henwood *et al*., 2002). These microorganisms may acquire resistance to many antimicrobial agents. During the last decades, hospital-acquired infections involving multi-resistant *Acinetobacter* sp. isolates have been reported, often in association with contamination of the hospital equipment or cross-contamination by the colonized hands of patient attending personnel (Bergogne-Bérézin *et al*., 1987; Bergogne-Bérézin and Towner, 1996b; Stone and Das, 1985; Struelens *et al*., 1993; Tankovic *et al*., 1994).

Tea from the leaves of plant Camellia Sinensis, next to water, is the most widely consumed beverage in the world. Depending upon the level of fermentation, tea can be categorized into three types: green (unfermented), oolong (partially fermented) and black (highly to fully fermented). Green tea has been found to be superior to black and oolong tea in terms of antioxidant, anti-inflammatory, anti-carcinogenic and antibacterial activity and other health promoting benefits (Siddiqui *et al*., 2006; Tiwari *et al*., 2005). Also green tea components show antiviral and antifungal activities in addition to their inhibitory effects on exotoxins (Taguri, 2004). Various studies have shown significant suppressive effects of green tea against many microorganisms, for example *Salmonella typhimurium* (Shetty *et al*., 1994), *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Plesiomonas shigelloides*, *P. aeruginosa* and many other species of bacteria (Toda *et al*., 1989; Taguri *et al*., 2004; Yam *et al*., 1997; Kim *et al*., 2004; Stapleton *et al*., 2004; Lee *et al*., 2003; Yam *et al*., 1998).

Many strains of *Acinetobacter* sp. shows high rate of resistance to several antibiotics and other kinds of antimicrobial agents (Henwood *et al*., 2002), so introducing of the new antimicrobial agents against this bacterium is one of the most important goals in treatment of such infections. However there is no study on investigation the antibacterial effects of green tea extracts on this bacteria.

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In this study we evaluated the antibacterial activity of water soluble green tea extracts on 20 hospital isolates of *Acinetobacter*.

MATERIALS AND METHODS

Bacterial strains and culture media: A total of 20 strains were collected from clinical specimens from burn wards of hospitals in Tehran, Iran. The isolates were further processed by the standard methods to identify as the *Acinetobacter* sp. Strains were maintained for long storage on Skimmed milk medium (BBL) by adding 10% glycerol in -70°C, cultures were maintained for daily use on Nutrient Agar (BBL) slants on 4°C. The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) medium (Pronadisa) were used for detection of antibiotic resistance of strains and measurement of the MBC of tea leaves extracts against each strain, respectively.

Preparation of tea leaves: Commercial green tea was used for doing experiments. Tea samples were stored in plastic bags at 4°C. Crude tea extracts were prepared by the method described by Tiwari *et al.* (2005).

Determination of antimicrobial activity of green tea extracts: The frozen bacterial strains were thawed and inoculated on nutrient agar medium and then cultured overnight at 36±0.5°C. The bacteria were suspended in 10 mL of sterile buffer saline and used as inoculate within 1 h after adjustment.

Two milligrams of the resulting green tea powder were dissolved in two mL of MHB. The solution was diluted serially in 8 stages.

Bacterial inoculate were added to serial dilutions of green tea, with final bacterial concentrations of (1.5×10^6 cell mL^{-1}). Antibacterial activity was measured by determining MBC by culturing on MHA medium in a sterile Petri dish. Five microlitre of each tube streaked on MHA plates, the highest dilution that inhibits bacterial growth on MHA after overnight incubation was taken as MBC (Tiwari *et al*., 2005; Sahm and Weissfeld, 2002).

Determination of the strains sensitivity to antibiotics: The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method (Bauer *et al*., 1966). To represent the different classes of antimicrobial agents commonly used for the treatment of *Acinetobacter* sp., we used piperacillin, Gentamicin, Ofloxacin, Ceftriaxone, Ciprofloxacin, Cephalotin, Ticarcillin, Kanamycin, Imipenem, Amikacin, Co-Trimoxazole, Ceftizoxime, ceftazolin, Carbenicillin (Hi-media, Mombay, India).

RESULTS

A total of 20 *Acinetobacter* sp. isolates were collected from specimens submitted to the clinical microbiology laboratories of selected hospitals in Tehran, Iran. Strains were isolated from burn wounds of admitted patients.

Sensitivity of Bacterial Species to green tea water soluble extracts: The average MBCs of the water soluble green tea extract against all strains of *Acinetobacter* sp. were 387.5±127.6 μg mL^{-1} (Table 1).

Sensitivity of bacterial species to antibiotics: The rates of resistance to different antibiotics for 20 studied isolates were shown in Fig. 1. Seventy five percent of isolated strains showed resistance to at least 12 antibiotics or

Table 1: The average of Minimum bactericidal concentrations for isolated strains of *Acinetobacter* sp.

No. of strains	MBCs for each strain (μg mL^{-1})
11	500
9	250
Total = 20	Average of MICs±SD = was 387.5 ±127.6 μg mL^{-1}

Table 2: Seven different Antibiotypes were recognized in 20 isolated strains. As shown, 45% of strains belong to Antibiotype 1 (only sensitive to Imipenem)

Antibiotypes	Percent
PIP^R G^R OF^R CI^R CF^R CH^R TI^R K^R I^S AK^R CO^R CK^R CZ^R CB^R	45
PIP^R G^R OF^R CI^R CF^R CH^R TI^R K^R I^S AK^S CO^R CK^R CZ^R CB^R	15
PIP^R G^R OF^R CI^R CF^S CH^R TI^R K^R I^R AK^S CO^R CK^R CZ^R CB^R	10
PIP^R G^R OF^R CI^R CF^R CH^R TI^SK^R $I^SAK^RCO^R$ CK^R CZ^R CB^R	5
PIP^R G^S OF^R CI^R CF^S CH^R TI^R K^R I^S AK^S CO^R CK^S CZ^R CB^R	5
PIP^R G^S OF^R CI^R CF^S CH^R TI^R K^R I^S AK^S CO^S CK^S CZ^R CB^R	15
PIP^S G^S OF^S CI^S CF^S CH^R TI^R K^S I^S AK^S CO^S CK^S CZ^R CB^R	5
Total	100

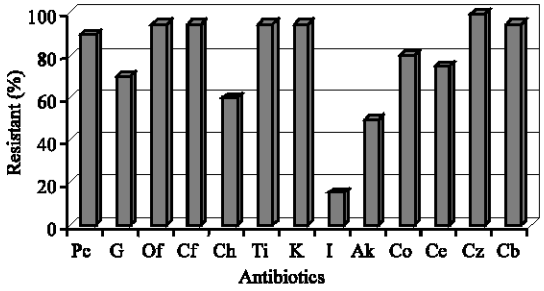

Fig. 1: Resistant percent to different antibiotics: piperacillin (Pc) 90%, Gentamicin (G) 70%, Ofloxacin (Of) 95%, Ciprofloxacin (Cf) 95%, Cephalotin (Ch) 60%, Ticarcillin (Ti) 95%, Kanamycin (K) 95%, Imipenem (I) 15%, Amikacin (Ak) 50%, Co-Trimoxazole (Co) 80%, Ceftizoxime (Ce) 75%, ceftazolin (Cz) 100%, Carbenicillin (Cb) 95%

more, all the strains were multi-drug resistant strains. Seven antibiotypes were recognized for all the isolated strains (Table 2). There is not any relations between sensitivity to green tea extract and antibiotypes in isolated strains.

DISCUSSION

The control of hospital acquired infection caused by multi-drug resistant gram negative bacilli has proven to be a particular problem in under developing as well as developed countries.

In the past few decades there was an increase in the importance of strictly aerobic gram negative bacilli, including *P. aeruginosa* and *Acinetobacter* sp.

Acinetobacter Play a significant role in colonization and infection of patients admitted to hospitals. They have been implicated in a variety of nosocomial infections, including bacteremia, urinary tract infection, secondary meningitis and nosocomial pneumonia. Treatment of such infections are often extremely difficult because of high resistance of these bacteria to the major groups of antibiotics, on the other hand, *Acinetobacter* sp. have a significant capacity for long-term survival in the hospital environment with enhanced opportunities for transmission between patients (Bergogne-Bérézin and Towner, 1996b), so it seems reasonable to explore new sources of natural compounds with antibacterial activity against *Acinetobacter* sp.

It was proven that green tea has anticancer and anti-hypercholesterolic activities, it has also anti bacterial activity that includes inhibition of gram positive cocci, gram negative bacilli and resistant strains such as vancomycin-resistant enterococci and methicillin resistant *Staphylococcus aureus* (Hamilton-Miller, 1995), as well as multi-drug resistance *P. aeruginosa* (Lee *et al*., 2003; Hosseini Jazani *et al*., 2006).

In this study isolated *Acinetobacter strains* showed high resistance to different groups of antibiotics (Fig. 1), however our results showed that green tea extracts had bacteriostatic as well as bacterioside effects on burn isolated *Acinetobacter* sp. (Table 1).

Our previous study showed that multi-drug resistance hospital isolated *P. aeruginosa* showed sensitivity to water soluble green tea extracts. The average MICs and MBCs against all strains of *Pseudomonas aeruginosa* were 2.06±1.76 and 2.54±2.22 mg mL^{-1}, respectively (Hosseini Jazani *et al*., 2006), however the present study showed that the concentrations needed for inhibition and killing of burn isolated *Acinetobacter* sp. is approximately 6.55 and 5.3 folds less than the concentrations needed for inhibition and killing of *P. aeruginosa* strains.

Present study is the first study that examined the antibacterial effects of green tea on the burn isolates of *Acinetobacter* sp., the present study shows that water soluble green tea extracts has significant activity with bactericidal action on multi-antibiotic resistant strains of *Acinetobacter*. In conclusion green tea is a safe agent may be used as an anti- *Acinetobacter* agent against antibiotic resistant strains. However more studies on numerous strains of *Acinetobacter* isolated from different clinical specimens as well as *in vivo* studies should be done for confirmation of the antibacterial effects of water soluble green tea extracts for treatment of *Acinetobacter* caused infections.

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