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Antibacterial Activity of Five Medicinal Plants Against Antibiotic Resistant Strains of Staphylococcus Aureus and Klebsiella Pneumonia

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Abstract

Antibiotic resistance is a considerable problem in the managing bacterial infections. Gram negative bacterium Klebsiella pneumoniae and Gram positive bacterium Staphylococcus aureus were screened for their resistance to five antibiotics. The concentration of antibiotics used was 10µg/ml. Both of the bacteria were found to resist antibiotics giving different patterns. The minimum inhibitory concentration (MIC) of these antibiotics against these bacteria was found to be around (10.0µg/ml to 0.3125µg/ml). The antibacterial activity of aqueous, cow urine and methanol extracts of Cinnamomum zeylanicum, Coriandrum sativum, Cuminum cyminum, Nicotiana tabacum and Syzygium aromaticum was also determined against antibiotic resistant strains. Our studies revealed that methanol extracts of the selected plants exhibited considerable activity against the test microorganisms. Thus it can be concluded that plants can be used as the potential candidates for developing new pharmaceuticals even against antibiotic resistant strains.

Keywords: Antibiotics, Antibiotic resistance, Plant extracts.

Introduction

Antibiotics are an indispensable part of modern medicine. Different antibiotics have different mode of action on different pathogenic organisms. The development and spread of resistance to antibiotics is a major problem. The increasing phenomenon of attaining resistance among microorganisms to antibiotics is accredited to the random and overuse of antibiotics (Usha et al., 2010) Today, clinically important bacteria are characterized not only by single drug resistance, but also by multiple antibiotic resistances because of the antibiotics misuse (Levy, 2002). Antibiotic resistance presents a global health threat (Stuart & Bonnie, 2004*). Worldwide emergence of Escherichia coli, Klebsiella pneumoniae, Haemophilus and many other ß-lactamase producers has become a major therapeutic problem.* About 70 percent of the bacteria that cause infections are resistant to at least one of the antibiotics most commonly used for treatment. *S. aureus* is a facultative anaerobic Gram-positive coccal bacterium that is frequently found in the human respiratory tract and causing skin infections and food poisoning. Around 90–95% of S. aureus strains worldwide are resistant to penicillin (Casal et al., 2005) and in most of the Asian countries 70–80% of the same strains are methicillin resistant (Chambers, 2001). The emergence of antibiotic-resistant forms of pathogenic S. aureus (e.g. MRSA) is a worldwide problem in clinical medicine. K. pneumoniae is a Gram-negative, non-motile, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines but can cause destructive changes to human lungs if aspirated. New antibiotic resistant strains of K. pneumoniae are appearing. The percentage of carbapenem-resistant K. pneumoniae (CRKP) is increasing and well documented (Naas, 2005). Few antibacterial therapy options exist for infections caused by CRKP. The changing pattern of antibiotic resistance has underscored the need for new antibacterial agents. The use of plant compounds for pharmaceutical purposes has gradually increased. Today, about 40 percent of our prescription medicines come from plant extracts or synthesized plant compounds. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Boer et al., 2005).

The present work deals with the antibiotic resistance of gram-negative and gram-positive bacteria and evaluation of the antibacterial potential of five plants against antibiotic resistant bacterial strains.

Materials and methods

Microorganisms

The selected microorganisms were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. The

gram-positive bacterium studied was *S. aureus* (MTCC 3160). The gram-negative bacterium was *K. pneumonia* (MTCC 4030). *S. aureus* and *K. pneumoniae* were maintained on Nutrient Agar and Luria Bertani (LB) medium respectively.

Antibiotics

Antibiotic discs and the antibiotics Ampicillin, Chloramphenicol, Erythromycin, Tetracycline and Trimethoprim were purchased from Hi Media Pvt. Ltd.

Plant material and extract preparation

Five plants viz *Cinnamomum zeylanicum* (bark), *Coriandrum sativum* (seeds), *Cuminum cyminum* (seeds), *Nicotiana tabacum* (leaves) and *Syzygium aromaticum* (flower buds) were used *based on traditional applications and pharmacological reports. The plant materials were collected from herbal gardens, surroundings and local market.* Further identification and authentication of the specimens *was done from Wild Life Institute of India, Dehradun.* The plant materials were washed thoroughly with distilled water and allowed to dry under shade for 7 days. The dried plant materials were grinded to powder. The powdered plant parts (10 g) were separately soaked in methanol, deionized water, and Cow urine (100 ml) in a clean and dry reagent bottle covered with a lid at 37 °C for overnight. The extraction was done by hot extraction method. The extracts were stored at 4 °C till further uses.

Screening for antibiotic resistance/susceptibility:
Disk diffusion assay

The screening was performed by using the agar disk diffusion method (National Committee for Clinical Laboratory Standards [NCCLS] , 1997). The petri dishes were prepared by pouring 25 ml of sterilized molten Nutrient agar/ LB media. The media was allowed to solidify. The selected bacterial strains (5×10^6CFU/ml) were cultured on Nutrient Agar/ Luria Bertani by using spread plate technique. Sterile antibiotic disks (6 mm) of five antibiotics studied were placed on the surface of the agar plates. All plates were incubated for 24 hours at 37°C. The zone of inhibition appearing around the disks was measured. All the tests were performed in triplicates and the mean values of the diameter of inhibition zones ± standard deviations were calculated. Further MIC of those antibiotics to which bacteria were susceptible was determined.

Antibacterial activity of plant extracts:
Agar well diffusion assay

Agar well diffusion method (Perez et al., 1990) was used to determine the susceptibility of the organism to the plant extracts. A loop full of the test organisms was activated by inoculation in 20 ml of nutrient broth. Nutrient agar media and LB media were used for evaluating antibacterial activity. The media was solidified and the test bacterial strains (5×10^6CFU/ml) were cultured by pour plate method. Wells were bored in the seeded agar plates with the help of a sterile aluminum borer (8.0 mm). 100 µl of the plant extract (30 mg/ml in dimethyl sulfoxide [DMSO]) was loaded into the well and the plates were incubated at 37 °C for 24 h. DMSO was used as a negative control. The microbial growth was determined by measuring the diameter of the zone of inhibition in millimeters appearing around the well.

Minimum Inhibitory Concentration (MIC)

MIC was determined by micro dilution technique as described by the National Committee for Clinical Laboratories standards (2000) (NCCLS) . Minimum inhibitory concentration (MIC) was determined for susceptible antibiotics and the plant extract showing antibacterial activity against test microorganism. A series of culture tubes were prepared all containing the 5 ml of nutrient broth inoculated with test microorganisms and incubated at 37°C. The final inoculum size was of approximately 5×10^6 CFU/ml (0.5 McFarland) (Farland, 1987). Decreasing concentration of antibiotics and the plant extract was added to the tubes; usually a step wise dilution (2-fold serial dilutions) was used. One tube was left without antibiotic and plant extract to serve as positive control and other without antibiotic, plant extract and inoculums to serve as negative control. The cultures were incubated for 24 hours at 37°c. The activity was measured as a function of turbidity at 660 nm. Lack of turbidity was further confirmed by pouring suspension aliquot of 0.1 ml into pre-sterilized Petri dishes with nutrient agar medium. The tests were performed in triplicates.

Results and Discussion

Antibiotics resistance in microorganisms: We determined the antibiotic resistance in *K. pneumoniae* and *S. aureus* strains to commonly utilized antibiotics. The results revealed that the microorganisms showed antibiotic resistance with varying magnitudes. The antibacterial activity of five antibiotics against two bacterial strains is summarized in Table 1. The zone of inhibition above 10 mm in diameter was considered as bacteria susceptible to antibiotics. Both of the tested organisms were resistant to one or the other antibiotics. Out of the five antibiotics tested, both of the bacteria were resistant to ampicillin. *K. pneumoniae* was susceptible to all other test antibiotics. *S. aureus* was resistant to erythromycin and trimethoprim but susceptible to chloramphenicol and tetracycline. Chloramphenicol and tetracycline showed antibacterial activity against both bacterial species tested. The gram-positive bacterium was resistant to multiple antibiotics while the gram-negative bacterium was resistant to single antibiotics. Although earlier studies indicated that gram-negative bacteria are more resistant to the antibiotics than gram-positive bacteria. Earlier studies also indicate that *K. pneumoniae* and *S. aureus* 'escape' the effects of many antibacterial agents (Boucher et al., 2009). The minimum inhibitory concentration range (determined by micro dilution assay following 24 h incubation) of the test antibiotics against the *K. pneumoniae* was 10.0–0.3125 *µg/ml*, whereas the range of the test antibiotics for *S. aureus* strains was 10.0-5.0 *µg/ml*. *K. pneumoniae* had more susceptibilities to antibiotics in comparison to *S. aureus*. There was no inhibition of growth with the vehicle control (10% DMSO).

Table 1: Antibiotic resistance
ZOI (mm) and MIC of antibiotics (µg/ml) against *S. aureus* 3160 and *k. pneumoniae* 4030

S.No.	Antibiotics	ZOI(mm) and MIC (µg/ml)	
		S.A	K. P
1.	Ampicillin	R	R
2.	Chloramphenicol	12.33±0.57 (5)	15.33±0.57 (2.5)
3.	Erythromycin	R	22±1 (0.3125)
4.	Tetracyclin	15.66±0.57 (10)	15±1 (10)
5.	Trimethoprim	R	20.66±0.57 (5)

Values are means of three determinations ± SD
S.aureus (S.A), *K.Pneumoniaee* (K.P), Resistant (R), MIC ( )

Out of the fifteen extracts (water, cow urine, methanol extract) of the five plants screened for their antibacterial activity against *S. aureus* and *K. pneumoniae* methanol extracts showed more antibacterial activity as compared to the water and cow urine extract. Out of the five plants screened more significant activity was shown by the methanol extract of *Syzygium aromaticum.* Among the five plants *Cuminum cyminum* and *Syzygium aromaticum* were more effective in comparison to *Coriandrum sativum, Cinnamomum zeylanicum, Nicotiana tabacum.* No antibacterial activity was shown by *Coriandrum sativum.* The range of MIC of the plant extract was 15- 3.75 mg/ml. Further MIC values also confirm that *Syzygium aromaticum* is most effective in comparison to other plants.

Table 2. ZOI (mm) and MIC of plant extracts (mg/ml)

against *Staphylococcus aureus* 3160 and *klebsiella pneumoniae* 4030

S .No	Plants	Water Extract		Methanol Extract		Cowurine Extract	
		S.A	K. P	*S.A*	K. P	*S.A*	K. P
1.	Coriandrum sativum	-	-	-	-	-	-
2.	Cuminum cyminum	15.33±0.57 (15)	-	15.66±0.57 (7.5)	-	15±1 (7.5)	-
3.	Cinnamomum zeylanicum	-	-	-	13.33±1 (15)	-	-
4.	Nicotiana tabacum	-	-	-	16.66±1 (15)	-	-
5.	Syzygium aromaticum	14.33±1 (3.75)	-	15.66±0.57 (3.75)	16.33±1 (3.75)	-	-

In summary, the bacterial species evaluated were resistant to ampicillin, erythromycin and trimethoprim but susceptible to chloramphenicol and tetracycline. The gram-positive bacterium exhibit more resistance in comparison to the gram-negative bacterium. *Cuminum cyminum* and *Syzygium aromaticum* showed considerable antibacterial activity.

Conclusion

From this study it can be concluded that the two microorganisms exhibited resistance to the various antibiotics studied. The present investigation provides support to the prevalence of antibiotic resistance among both gram-positive and gram-negative bacterial strains. Thus, there is a need for antibiotic policy in a health care setting which should include addition of newer antibiotics, raising awareness regarding the disadvantages of irrational use of antibiotics, availability of antibacterial agents only through prescription provided by licensed and qualified health care professionals, be familiar with the basic principles and procedures of the standard screening protocols ant the protocols should be updated every 2-3 years, in view of the rapidly changing antibiotic resistant pattern. Further it is also concluded that medicinal plants also offer considerable potential for developing new antibacterial .As *the problem of antibiotic resistance is growing so further more plants should be investigated.* Hence, research should be focused towards this direction to identify more medicinal plants which exhibit significant antibacterial activity.

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