A study of the efficacy of eugenol on Acinetobacter species

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Acinetobacter is commonly isolated bacterium in clinical samples isolated from upper respiratory tract patients subjected who are subjected to antibiotics. Resistance has become a common feature in this bacterium due to uncontrolled use of 3GCs- third generation cephalosporin's -cefotaxime, ceftazidime, ceftriaxone) as they are commonly used for treating Gram negative infections. Clove (Syzygium aromaticum) possesses antibacterial properties which restricts the growth of antibiotic resistant organism.

Introduction

Acinetobacter is the most frequently isolated pathogen in clinical specimens. It has a wide spread etiology and is found associated with many of infections, including nosocomial pneumonia, meningitis, endocarditis, burns, skin and tissue infections. *Acinetobacter baumannii is* a frequent isolate from Gram-negative sepsis in immune compromised patients, posing risk of high mortality *(Kopronova et al., 2001). Wisplinghoff et al.,* (2000) have shown that the outbreaks of infection with contaminated respiratory equipment, bedding materials and transmission mediated through hospital personnel linked to *Acinetobacter* species. During recent years, *Acinetobacter baumannii* has become a significant pathogen, especially in intensive care units. *Peleg et al.,* (2008) have described this organism as emerging important pathogen. Its ability to chronically colonize patients and cause outbreaks that are usually hard to eradicate poses significant challenge to restrict and there by results in the increase of healthcare expenditure. In addition to its intrinsic resistance to many commonly used antibiotics, this organism g has the ability to gain additional resistance rapidly in response to newer antibiotics introduced for treatment. *(Neonakis et al., 2011).*

Secondary plant metabolisms include products that aid in the growth and development include a range of compounds from different metabolite families. These compounds are essential for the maintenance of life of the plant, and are also involved in plant protection against biotic or abiotic stress *(Hattenschwiler and Vitousek, 2000).* These mainly include Terpenes-- derived from the C5 precursor isopentenyl diphosphate (IPP), Alkaloids--derived from amino acids, Phenolics--shikimate pathway or malignant/acetate pathway.

In alternate medicine, clove is also used as an expectorant, as it makes it easier to cough up phlegm. Chewing combination of cloves with pepper is very beneficial in upper respiratory infection. Clove is very effective in relieving the symptoms of chronic cough in Pertussis. A decoction of Clove oil, honey and garlic is a very effective in giving relief from debilitating cough associated with Asthma and Tuberculosis. Clove oil expectorant capabilities have proved useful in various respiratory disorders, including bronchitis, colds, sinusitis, asthma, and tuberculosis. Chewing a Clove bud eases sore throats. The anti-inflammatory effect of the fumes from the Clove oil loosen respiratory passages, and also clears the nasal tract, thereby improving breathing and treating congestion and other respiratory problems *(Jadhav et al., 2004). The oil obtained from Clove possesses antibacterial properties and used in various dental creams, toothpastes, mouthwash, and throat spray to cleanse bacteria. It is also used as pain reliever and cure for sore gums to improve overall oral health. Due to its antiseptic characteristics, it is an effectively used for treating common problems such as cuts, fungal infections, burns, insect bite etc. Clove oil has proved as a strong antifungal agent against opportunistic fungal pathogens such as Candida albicans, Cryptococcus neoformans and Aspergillus fumigates (Dorman and Deans, 2000; Ahmad et al., 2005; Pawar and Thaker 2009)*

ESBL-EXTENDED SPECRTUM BETA LACTAMASES; The extensive use of third generation cephalosporins as a first line of treatment in hospital set up leading to the development of resistance towards cephalosporins. Until 1980 resistance to β-lactam antibiotics were known to be limited to organisms with inducible chromosomal β-lactamase genes (in this form the resistance in not transportable).

Consequently, it came as an unwelcome surprise when species of *Klebsiella* with plasmid mediated resistance was isolated in Germany in1983 *(Vuye A, 2012)*. β -lactamase are a group of enzymes act upon β -lactam antibiotics (antibiotics have a four carbon atoms ring, called as β –lactam) and render them ineffective *(Kirstein P 2008*).NDM-1 (New Delhi Metallo-beta-lactamase-1) was initially detected in a *Klebsiella pneumoniae* strain which was isolated from a Swedish patient of Indian origin in 2008. It was later detected in isolates in India, Pakistan and the United States, is one of the newly developed resistant strain in this group *(Kumaraswamy et al., 2010).*

Material & Methods

i. This prospective study was conducted in the Department of Microbiology, T.N.M.C.186 isolates were obtained from clinical samples.
ii. Phenotypic identification and confirmation of Acinetobacter, species was done using analytical profile index or API system for identification of clinically relevant isolates.
iii. Antimicrobial susceptibility testing: Kirby Bauer disk diffusion method, documented by The National Committee for Clinical Laboratory was used to test the antibiotic sensitivity (NCCLS). Antibiotic discs from Hi -media laboratories *for Gram Negative- Dodeca Enterobacteriaceae-1 DE053- of* mentioned antibiotics and known concentrations were used along with testing of quality control strains of *E. coli ATCC 25922* and *Pseudomonas aeruginosa ATCC 27853. was used to determine the antibiogram of the Acinetobacter isolates*
iv. HPTLC fingerprinting profile**:** As herbal extracts are containing many constituents; it is important to confirm the bioactive compound. It is critical to obtain reliable chromatographic fingerprint which represents and helps in identification of the pharmacologically active compound and helps to know the chemical characteristic of the components present in the extract. The HPTLC fingerprinting profile is an important parameter of herbal drug standardization especially in cases of identification of the bioactive component in medicinal plants. The extracts along with the standard markers were subjected to HPTLC. Markers served to calculate the quantity of herbal substances or herbal preparations in the herbal product.

Results

i. 186 samples showing mono microbial growth were considered as probable or confirmed pathogens and were used
ii. Acinetobacter isolates were obtained from respiratory samples,12 (92.3%) were found to be producing ESBL by the DDST.
iii. Of the 13 isolates chosen for the disk potentiation test, of which 5 isolates were found ESBL-positive by the DDST. Cefepime and cefotaxime together with the clavulanate was used.
iv. All isolates were subjected to antibacterial susceptibility testing to Aqueous and alcoholic extracts of clove
v. HPTLC fingerprinting profile

Instrument application	CAMAG Linomat 5	**Sequence**	
Spray gas	Inert gas	Syringe size	100 µl
sample solvent type	methanol	Number of tracks	5
Dosage speed	150 nl/s	Application position	8.0 m.m.
Pre dosage volume	0.2µl	Band length	8.0 m.m.
Application position	8.0 m.m.		
Solvent front position	80.0 m.m.		

DETECTION PARAMETERS- Lamp: D2/ Wave length: 254 nm

Tracks	**1**	**2**	**3**	**4**	**5**
Applied sample	Aqueous extract		Std Eugenol	Alcoholic extract	
Applied volume	5µl	10µl	5µl	5µl	10µl
R_f	---	--	0.46	0.45	0.44
Area of the peak	--	--	13946.6	1901.4	3292.1

DTECTION PARAMETERS- Lamp: Hg/ Wave length: 366 nm

Tracks	**1**	**2**	**3**		**4**	**5**	
Applied sample	Aqueous extract		Std Eugenol		Alcoholic extract		
Applied volume	5µl	10µl	5µl		5µl	10µl	
R_f	--	--	0.11	0.46	--	0.04	0.45
Area of the peak	--	--	967	1710.4	--	201.6	627.9

The presence of Eugenol in the methanolic extracts was confirmed as R_f 0.46 was seen in standard as well as in methanol extract. UV absorption spectra also showed similar peaks of maximum absorption in extract and standard Eugenol

Comparison of zone of inhibition between Aqueous and Alcoholic Extracts (*n=186)*

Variables	**Extracts**	**Mean**	**SD**	**Median**	**IQR**	**t-value**	**p-value**
CL ^		12.06	3.41	12.00	4.00	-10.618	2.47E-26
	Alcoholic	21.41	6.547	22.00	10.00	Difference is significant	

Conclusion

Acinetobacter is found to be increasingly associated with nosocomial infections; they are most often associated with infections of the respiratory system. Studies in India have reported high levels of resistance in Acinetobacter's. Acinetobacter isolates were from the critical care setting and there was a high level of resistance to the majority of the antibiotics tested. In our study, they were 6.8 %of the total isolates s (n=13) of which (n=12) 92.3% were ESBL-producing This relatively high prevalence of ESBL in Acinetobacter isolates in the present study can be attributed to selection pressure in the microbial community due to extensive use of antibiotics. Cefepime was the most sensitive in detecting ESBLs when the cephalosporins were tested. Clove proved to be effective in limiting the growth of these species, with a mean zone of inhibition of 14.44 (aqueous extracts) and 29.50 (alcoholic extracts). Indicating alcoholic extracts to be highly effective, as the zone of inhibition was much higher than most of the conventional antibiotics used for treating these infections. Clinical isolates of Stenotrophomonas (Pseudomonas) maltophilia and Acinetobacter isolate which showed resistances to multiple antibiotics were successfully inhibited by Clove extracts. These results were noteworthy as controlling such drug resistant strains is a great challenge in chemotherapy. Clove in alcoholic extraction showed the highest growth inhibitory effect against micro-organisms with maximum ZOI of 38.0 mm.

Discussion

Definitive identification and characterization of ESBL can be confirmed by molecular techniques. The study mainly intended to analyse the ESBLs in Acinetobacter, wherein high level of resistance was detected in Acinetobacter species to most antibiotics tested. Judicious use of antibiotics, especially in the ICUs, and appropriate infection control measures are necessary to control the spread of such infection in hospitals needs to be focused primarily *Clove can be of great rescue as the extract does not contain a single compound, but a mixture of complex compound (Eugenol, Eugenyl acetate, methyl salicylate, beta-caryophyllene, 2-heptanone, acetyl- eugenol, , iso-eugenol, methyl-eugenol, dehydrodieugenol, kaempferol, rhamnocitrin, , gallic acid, ellagic acid, myricetin and oleanolic acid .Developing resistance is a difficult trial for bacterial strains.*

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