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ANTIBACTERIAL ACTIVITIES OF SOME ANTIBIOTICS AGAINST CLINICAL ISOLATES OF CERTAIN BACTERIAL STRAINS WITH SPECIAL REFERENCE TO *Lantana camara* LEAF EXTRACTS

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ABSTRACT

Fifteen clinical isolates comprising of *Corynebacterium acnei* (03), *Escherichia coli* (04), *Klebsiella pneumoniae* (03) and *Staphylococcus aureus* (05) were collected from Gandhi Medical College, Bhopal, India and their resistant pattern against four well known antibiotics; ciprofloxacin (CIP), erythromycin (E), gentamicin (GEN) and methicillin (MET) (all 10 µg disc^{-1}) were studied using disc diffusion method with *Lantana camara* leaves extract. *Staphylococcus aureus* (80% against MET and 60% against GEN) and *Escherichia coli* (75% against MET and 50% against E), in case of *Klebsiella pneumoniae* (66.67% against MET, GEN and CIP) and *Corynebacterium acnei* (66.67% against MET) species were found to be more resistant against the studied antibiotics as compared to the extracts of *Lantana camara*. No resistance was observed of *Lantana camara* leaves derived antibiotics against all the test isolates. It is concluded from these figures that microbial resistance against these antibiotics are increasing in our population which is alarming and therefore it is recommended to physicians to prescribe these antibiotics unless no other substitute is available in clinical practices.

KEYWORDS: Antibiotics, *Corynebacterium acnei, Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus, Lantana camara*

Many bacteria are responsible for diseases of man, plants and domestic animals. The host has got its own defense mechanism to fight the trouble-making microbes, in other words the host mobilizes all 'antibacterial measures' at its command. The life and health of human beings, animals and plants are constantly threatened by parasites of all kinds, especially by microorganisms. During the last century, numerous microorganisms (pathogens) have been recognized, and various effective methods of prevention and treatment have been discovered. We are constantly in contact with the microorganisms in the environment.

All forms of biological associations between microorganisms and animals are known. Microorganisms exist in loose associations as commensals as mutualists, as parasites and as pathogens causing organisms, got a boost when a German pathologist Rudolf Virchow discovered that microorganisms cause many diseases of animals and human. Among the various infectious diseases, the bacterial and fungal diseases are common in human beings. Leprosy (*Mycobacterium leprae*), tuberculosis (*Mycobacterium tuberculosis*), pneumonia (*Diplococcus pneumonoeae*), cholera (*Vibrio cholarae*) etc. are some common bacterial diseases of man. Likewise candidiosis (*Candida albicans*), Tinea pedis, Tinea corporis, Tinea capitis, Tinea manus and Tinea cruris (*Microsporum Sp.*, *Trichophyton* and *Epidermophyton*) etc. are fungal diseases of human.

Antimicrobial resistance is an increasing problem that contributes to morbidity, mortality and increased health care cost with tremendous variability not only amongst pathogens causing various clinical infections in different geographic regions, but also over time in specific areas (Hsueh *et al.,* 2008). Asia Pacific region has been the area with the highest levels of antimicrobial resistance amongst the five global regions. Human misuse of antibiotics plays a major role in resistance. Resistance means that an organism ceases to be killed or inhibited by a drug. This problem may occur when antibiotics are used in every disease. It is a common practice that many patients discontinue antibiotic therapy as soon as they feel better irrespective of the outcomes. These aborted treatments encourage drug resistance. Another source of resistance against antibiotics is animals.

Although medical practices are flourishing very fast in this era, yet many diseases are there that needs

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suitable agents to get cured. Due to growing resistance, many bacterial infections even today are not being treated effectively. If these infections are not treated properly then they may become fatal threat in the future (Paustian, 1999). Among the antimicrobial agents, the development of the modern antibiotics has vastly improved the treatment of cutaneous bacterial infections and is widely prescribed to treat various diseases. Like other antibiotics, resistance by organisms has also been reported by many workers in other parts of the world. Resistant pathogens are associated with higher morbidity and mortality than those caused by susceptible pathogens. Therefore, the global impact of increasing resistance is a major concern.

The objective of the present work was to determine the resistance pattern and bactericidal action of four antibiotics namely ciprofloxacin, erythromycin, gentamicin and methicillin and *Lantana camara* leaves ethanolic extracts by disc diffusion method against fifteen clinical isolates comprising of *Corynebacterium acnei*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection and Maintenance of Clinical Isolates

Clinical isolates were collected from Department of Microbiology, Gandhi Medical College, Bhopal, India. They are identified as *Corynebacterium acnei (03)*, *Escherichia coli (04)*, *Klebsiella pneumoniae* (03) and *Staphylococcus aureus (05)*. All the bacterial isolates were grown in nutrient broth at 37˚C and maintained with subcultures on nutrient agar (HiMedia make) slants at 4˚C.

Preparation of Culture Media

Nutrient broth and nutrient agar media were prepared for maintenance of bacterial isolates and Mueller - Hinton agar was prepared for antibacterial activity. These mediums and broth were prepared and sterilized according to manufacturer's instructions (HiMedia).

Antibacterial agents

Standard discs of antibiotics namely ciprofloxacin (CIP), erythromycin (E), gentamicin (GEN) and methicillin (MET) (all 10 µg $disc^{-1}$) were purchased from HiMedia.

Preparation of *Lantana camara* Leaves Ethanolic Extracts and Plant Derived Antibiotic Disc (Lc)

Plant extracts were prepared by the method of Harborne (1984). Briefly, 100 grams of powered plant sample was extracted with 100 ml of ethanol using the Soxhlet apparatus. The crude extracts (100 mg) were dissolved in 1 ml of dimethyle sulphooxide (DMSO) and were filtered by using membrane (pore size 0.47 µm). The discs of 6 mm diameter (Sterile blank, HiMedia) were impregnated into the concentration of the extract. The final impregnated discs (Lc) used for the sensitivity test were 100 mg $disc^{-1}$. These impregnated discs were dried in incubator at 37 ºC for 18 24 hours and after this stored in an amber colour glass bottle at room temperature until further use.

Preparation of Inoculum

The inoculation was prepared by touching the top of the colonies of the isolates with sterile wire loop and suspending in a tube containing 3-5 ml of broth. The tubes are then incubated at 37°C for few hours.

Preparation and Inoculation of Plates

Mueller - Hinton Agar was poured into sterile Petri dish about 10-15 ml per plate. The plates were then allowed to solidify. After 15 minutes the inoculation of bacterial suspension was carried out. For this purpose, a sterile swab was dipped into a bacterial broth suspension. Excess fluid was removed by pressing and rotating the swab against the side of tube above the level of suspension. The swab was then streak evenly over the surface of the medium. After inoculation, surface of agar was allowed to dry.

Antibacterial Assay

For this assay, the disc diffusion method of Bauer et al., (1966) was used. The antibacterial agents of known potency and plant derived antibiotic discs were placed in triplicate on the agar surface by using sterile forcep and gently pressed down to ensure its contact with agar and the plates were incubated for 18 24 hours at 37˚C. After 24 hours of incubation, the plates were examined and zones of growth inhibition were measured surrounding the discs (including the 6 mm disc) with the help of meter scale. The end point was taken as complete inhibition of growth as determined by the naked eyes.

RESULTS

In the present study, resistant pattern of fifteen (15) clinical isolates of *Corynebacterium acnei* (03), *Escherichia coli* (04), *Klebsiella pneumoniae* (03) and

Staphylococcus aureus (05) were studied using antibiotics namely ciprofloxacin, erythromycin, gentamicin and methicillin (all 10 µg $disc^{-1}$) and *Lantana camara* leaves ethanolic extracts and the results are depicted in Table and Figures. The results revealed that 33.33% clinical isolates of *Corynebacterium acnei*, 40.00% *Escherichia coli*, 46.67% *Klebsiella pneumoniae* and 44.00% *Staphylococcus aureus* were resistant to all the antibiotics. All the 15 isolates were 40.00% resistant to ciprofloxacin and erythromycin. In case of gentamycin, the results showed that 53.33% clinical isolates were resistant. Similarly, 73.33% were resistant to methicillin.

In the case of ciprofloxacin only one isolate of *Corynebacterium acnei* and *E. coli* were resist whereas two isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus* were resist. Similarly in case of erythromycin, two isolates of *Escherichia coli* and *Staphylococcus aureus* were resist whereas only one isolate of *Corynebacterium acnei* and *Klebsiella pneumoniae* were resist. Two isolates of *Escherichia coli* and *Klebsiella pneumoniae* were resist whereas one isolates of *Corynebacterium acnei* and three of *Staphylococcus aureus* were resist against gentamicin. Similarly, two isolates of *Corynebacterium acnei* and *Klebsiella pneumoniae* were resist whereas three of *Escherichia coli* and four of *Staphylococcus aureus* were resist against methicillin. No resistance was observed against *Lantana camara* leaves derived antibiotics.

From these table and figures (1, 2, 3 and 4), it is clear that *Klebsiella pneumoniae* and *Staphylococcus aureus* are more resistant against all the antibiotics as compare to *Corynebacterium acnei* and *E. coli* but in case of *Lantana camara* there is no resistance were found. It

Table: Antibacterial activity of antibiotics and *Lantana camara* ethanolic extract

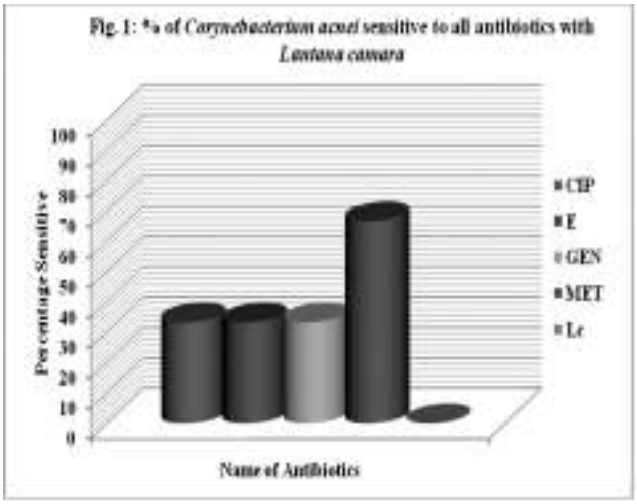
Bacteria[a]	Strain	ZOI and resistance against some antibiotics[b]					Total resistant in %
		CIP	E	GEN	MET	Lc	
C.a.	Ca 1	+2[c]	+1	--	--	+1	33.33
	Ca 2	+2	--	+2	+1	+2	
	Ca 3	--[d]	+1	+1	--	+2	
	% resis.	33.33	33.33	33.33	66.67	00.00	
E. coli	Ecoli 1	+3	+2	--	--	+2	40.00
	Ecoli 2	--	+2	+1	--	+1	
	Ecoli 3	+3	--	--	+1	+2	
	Ecoli 4	+1	--	+1	--	+2	
	% resis.	25.00	50.00	50.00	75.00	00.00	
K. p.	Kp 1	--	+2	--	--	+1	46.6 7
	Kp 2	--	+2	+2	--	+1	
	Kp 3	+1	--	--	+1	+2	
	% resis.	66.6 7	33.33	66.67	66.6 7	00.00	
S. a.	Sa 1	+2	--	--	--	+1	44.00
	Sa 2	+2	--	+2	--	+2	
	Sa 3	--	+2	--	--	+1	
	Sa 4	+2	+1	--	--	+1	
	Sa 5	--	+1	+2	+1	+1	
	% resis.	40.00	40.0 0	60.00	80.00	00.00	
Total resistant		06	06	08	11	00	--
Total isolates		15	15	15	15	15	--
% resistant		40.00	40.00	53.33	73.33	00.00	--

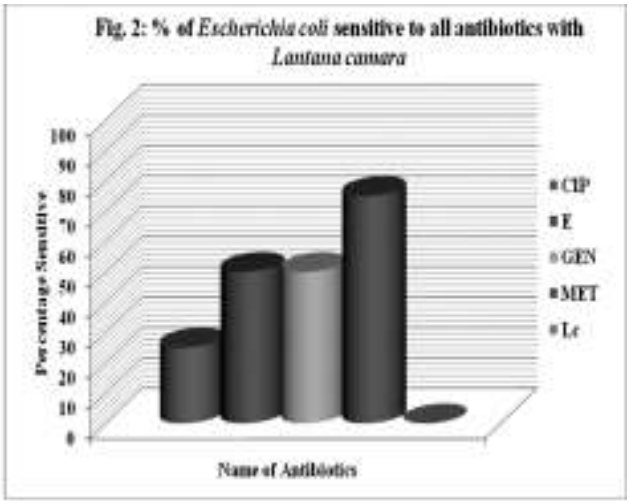
[a] *C. a. = Corynebacterium acnei*, *E. coli = Escherichia coli, K. p. = Klebsiella pneumoniae, S. a. = Staphylococcus aureus*
[b] CIP = Ciprofloxacin, E = Erythromycin, GEN = Gentamicin, MET = Methicillin
[c] +1 = 6 - 10 mm, +2 = 10 - 20mm, +3 = 20 – 30mm
[d] -- = No Zone of inhibition

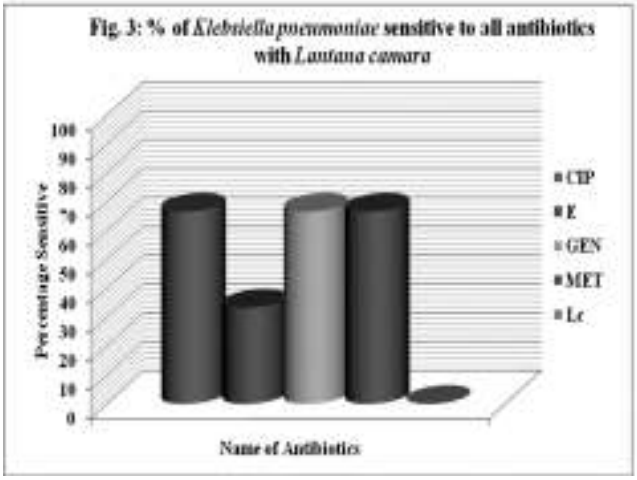
appears that the growth of all the clinical isolates were inhibited with *Lantana camara* leaf extracts. However, in case of *Staphylococcus aureus* very high resistance as compare to the rest of clinical isolates against methicillin (80%).

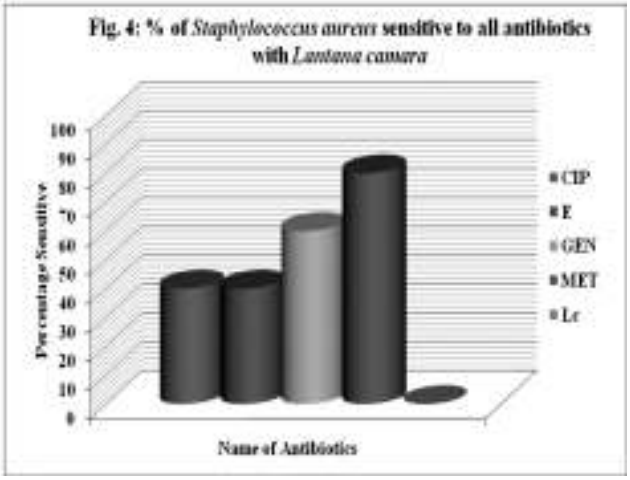
DISCUSSION

Major factor limiting the long-term use of antimicrobial agents is resistance. Before antibiotics era, many people died of bacterial infections caused by pathogens as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumonia*. Use, abuse or misuse of antimicrobial agents has encouraged the evolution of bacteria towards resistance that often results in therapeutic failure. Prescribing practice of specific class of antibiotics to certain organisms has been found to play a critical role in development of resistance against that antibiotic (Costelloe et al., 2010). Thus, antimicrobial resistance findings and understanding are necessary to help minimize the emergence of multi drug resistant organisms by promoting prudent use of antibiotics, for this purpose, the need for general public to be appropriately informed on use of antibiotics has been emphasized. Resistance to erythromycin is becoming a serious clinical problem. A study on prevalence of antimicrobial resistance among Gram-negative isolates in an adult intensive care unit at a tertiary care center shows decreased susceptibility of *E. coli* and *Klebsiella,* besides other organisms, to various antibiotics (Al-Johani et al., 2010). A recent data shows that hospital-acquired isolates of *K. pneumoniae*, rather than outpatient isolates, are more likely to be resistant to multiple antibiotics (Al-Tawfiq and Antony, 2007). In another work, resistance rates to three antimicrobials (ciprofloxacin, erythromycin and nalidixic acid) in Campylobacter isolated from organically and intensively reared chickens in London were taken into consideration.









Using preset breakpoints, all isolates from all groups of chickens were identified as resistant to erythromycin (Soonthornchaikul et al., 2006). The development of the modern antibiotics has vastly improved the treatment of cutaneous bacterial infections, particularly those caused by *Staphylococcus aureus*.

Ruhe et al., (2005), very recently reported that the resistance mechanism developed by the *Staphylococcus aureus* against tetracycline and methicilline. A wide range of antibiotics, which have been used in dermatological practice, show-increasing frequency of resistance. Therefore, today antibiotics can treat most bacteria causing skin diseases effectively. The indiscriminate use of antibiotics in some parts of the world in both human and veterinary medicine has led to the emergence of resistant strains of bacteria. Thus, the rational use of antibiotics is of utmost importance. This is why, the antibacterial therapy by medicinal plants will focus on a few problem areas.

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. Plant materials or their extracts have been utilized as drugs since long in many parts of the world, India is the oldest among them **(**Chopra et al., 1992**)**. Over the last few years a large number of plant species have been evaluated for their antibacterial activity (Juliana et al., 2002; Agrawal et al., 2007; Agrawal et al., 2012). At the same time, indigenous people of the rest of the world also utilize plants for curing diseases. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists (Kafaru, 1994). Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties.

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