ISCA Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(3),** 14-19, July **(2012)** *ISCA J. Biological Sci.*

Survey on Drug Resistant Pattern of Clinical Isolates and Effect of Plant Extract on the Drug Resistant Pattern

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Available online at: www.isca.in
Received 11th May 2012, revised 13th June 2012, accepted 17th June 2012

Abstract

Drug resistance is an emerging fact of the human beings leading to an increase in mortality rate. For this instance clinical samples were collected such as urine, pus, sputum, and catheter samples from private clinical laboratories, Madurai. The following organisms were isolated and characterized as E. coli (49/111), Klebsiella pneumonia (28/111), proteus mirabilis (8/111), pseudomonas aeruginosa (18/111) and staphylococcus aureus (8/111). Further these clinical isolates were subjected to SDS treatment or pomegranate extract treatment for plasmid curing and continued with antibiotic susceptibility test. In our present study, ceftriaxone was found to be more active than any other antibiotics. On the contrary, penicillin G was found to be inactive against all the clinical isolates. The plasmid DNA was separated by agarose gel electrophoresis in E. coli and pseudomonas aeruginosa. Thus resistant organisms, which was examined as multidrug resistance was taken into account for molecular characterization of gene responsible. The plasmids were cured in all the isolates by sodium dodeyl sulphate (or) pomegranate extract. There was no correlation between plasmid pattern and their antibiogram.

Keywords: Clinical isolates, multi drug resistance, pomegranate extract, plasmid curing.

Introduction

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs[1]. Drug resistance is more frequently encountered in hospital-acquired pathogens; however the incidence of antibiotic resistant pathogens in community-acquired infections has been also on the rise in recent years[2].

Antibiotic resistance is a global public health problem. Although all countries are affected, the extent of the problem in the developing nations is unknown. With increasing travel and patient movement throughout the world, transmission of drug-resistant organisms from one country to another became a possibility[3].

The R-plasmid mediated resistance is often and presumably with increasing frequency, multiple, which means that the R plasmid-carrying bacteria are resistant to 3 or 5 or even more antibiotics. There are also some indications of an increasing frequency of multiple resistances in especially virulent strains of different bacteria that produce plasmid-determined pathogenecity factors[4].

Multiple antibiotic resistances to useful classes of antibiotics including the beta-lactams, aminoglycosides and quinolones has generally emerged and this has been increasingly observed among a number of gram-negative pathogens such as the *Enterobacteriaceae* bacteria[5].

Pomegranate peels are exploited in traditional medicine because of their strong astringency, making them a popular remedy throughout the world. In the form of an aqueous decoction (i.e., boiling the hulls in water for 10-40 minutes), it was used for dysentary and diarrhea, and also for stomatitis it can be drunk, used as a mouthwash, douche or enema.

Pomegranate (*Punica granatum* L., Punicaceae), is one of the oldest known drug. Dried fruit peel is used for diarrhea and to treat respiratory and urinary tract infections. Also, pomegranate fruit peel exerted diverse pharmacological functions as antioxidant activity[6,7].

Antimicrobial activities of pomegranate have been studied by some researchers, and they reported that the pomegranate extracts was not show inhibitory effects against Gram-negative bacteria, including *E.coli*. On the contrary, other researchers, proved that pomegranate extracts have positive antibacterial activity against some bacterial strains, including *E.coli* and *S. aureus.* In a previous study[8], reported that pomegranate fruit peel compound punicalagin have antimicrobial activity against *S. aureus* and *P. aeruginosa*[8].

The extra chromosomal DNA called plasmids is capable of conferring resistance of microorganisms to drugs[9]. Based on this plasmid curing to presumptively link the observed resistance to vancomycin and other beta-lactam antibiotics on the multi discs used was carried out.

Based on the above gathered information, this work is mainly focused on plasmid curing of multidrug resistant bacteria isolates using pomegranate peel extract and to know the overcome method of antibiotic resistance.

Material and Methods

Sample Collection: Clinical isolates were collected from various clinical laboratories from Madurai. The bacteria were isolated from clinical samples such as urine, pus, sputum, and catheter samples. The bacterial identity was confirmed by staining and biochemical methods[10].

Antibiotics used: The antibiotics use ampicillin, ceftriaxone, amoxycillin, penicillin – G, co-trimaxazole, nitrofurantoin, ciprofloxacin, amikacin, gentamicin, chloramphenicol, streptomycin and tobramycin.

Antibiotics Sensitivity Testing: The antibiotic sensitivity testing of the isolates was done on Muller Hinton agar with Kirby-Bauer disk diffusion method, performed according to the recommendations of clinical laboratory standards institute-CLSI. Antibiotic impregnated paper discs of CLSI levels were obtained from Hi-media, India. Different set of antibiotic discs were used against different bacteria. Depending on the size of clear zone formation, the cultures were labeled as sensitive, moderately sensitive or resistant to the array of antibiotics used in the study[11].

Plasmid Analysis: Isolates having more than 70% resistance were selected for plasmid studies. Plasmid isolation was done using alkaline lysis method adopted from[12]. The isolated plasmid samples were interpreted by running them on 0.8% agarose gels. A single well was loaded with Molecular weight marker. After electrophoresis, the gels were visualized under UV illumination in a gel-documentation system.

Plasmid Curing: The plasmid curing was performed by introducing SDS and pomegranate peel extract treatment.

Preparation of Pomegranate peel extract: Pomegranate fruit peel purchased from local market was dried and powdered before extraction. Powdered plant material (500g) was repeatedly extracted with two litre solvents of increasing polarity starting with ethanol (80%) and distilled water. The percolation time for solvent was 24h. The extracts were filtered, concentrated and freeze dried. The residue yield from each solvent was stored at 4^0C[13].

Determination of sublethal concentration of pomegranate peel extract: The extract of *Punica granatum* L. exhibited inhibitory effect against *Escherichia coli*[14]. The sublethal concentration of pomegranate peel extract was derermineded by Minimum inhibitory concentration[15].

Plasmid curing with *sodium Dodecyl sulphite:* Sub-inhibitory concentration of sodium dodecyl sulfate (SDS) was used for plasmid curing. Antibiotic resistant isolates were grown at 37^0C for 24 hours in Nutrient broth containing 10% SDS. After which, the broth was agitated to homogenize the content and a loopful subcultured onto Mueller Hinton agar (MHA) plates. The plates were incubated at 37^0C for 24 hours[16].

Antibiogram Analysis (after plasmid curing): The colonies were screened for antibiotic resistance by the disk diffusion method. Cured markers were determined by comparison between the pre- and post- curing antibiograms of isolates. Loss of resistance markers gave an indication that those markers were probably located on a plasmid and not on the chromosome.

Results and Discussion

The clinical isolates were collected from various samples (urine, pus, sputum and catheter) obtained from clinical laboratories in Madurai. These organisms were isolated and characterized as *E.coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia*e (table- 2). The high rates of antimicrobial resistance reported among these bacterial pathogens[17,18]. The isolates were subjected in to antibiotic susceptibility test to identify the resistant and sensitive pattern of organisms. From the result, we conclude most of the antibiotics were found to be resistant for *E.coli*, similar result was obtained, ceftriaxone was found to be more active than any other antibiotics. On the contrary, penicillin G was found to be inactive against all the clinical isolates (figure 1-5). The isolates were treated with SDS and pomegranate peel extract for plasmid curing. The antibiotic susceptibility test performed before and after curing was compared with the standard chart[3]. The results showed that drug resistant gene transfer the genetic information in to various species of pathogens. In turn with above the prevalence of strain *E.coli* and *pseudomonas* sps were resistant for the above mentioned antibiotics. The isolates showed more similarities in their antibiogram but their plasmid profile is entirely different from each other. Similar results were described by[19]. The sublethal concentration of pomegranate peel extract was determined by minimal inhibitory concentration[20]. The minimal inhibitory concentration values are 0.78, 0.39, 0.19, 0.09, 0.02mg/ml respectively. The development of drug resistance in human pathogens against commonly used antibiotics necessited a search for new antimicrobials of mainly plant origin[21]. The plasmid curing and antibacterial activity of pomegranate peel extract showed good result against pathogens (table – 3).

Conclusion

Development of resistance in *Pseudomonas aeruginosa* and *Escherichia coli* is one of the problems. This resistance may be indiscriminate and inappropriate use of antibiotics. This calls for the education of both medical and paramedical staff on the rational use of antibiotics. *Escherichia coli* is the common bacterial species found in hospital environment. Development of antibiotic resistance in enteropathogenic *Escherichia coli* is also dangerous and may lead to epidemics.

The knowledge of susceptibility testing patterns of the bacterial strains will guide the clinicians to choose appropriate and judicious antibiotics for treatment of wound infections. Updating the antibiogram will further reduce the complications of resistance[22].

Furthermore, due to the presence of good antioxidant potential in this plant it is suggested that pomegranate may be included in the diet for a healthy lifestyle[23].

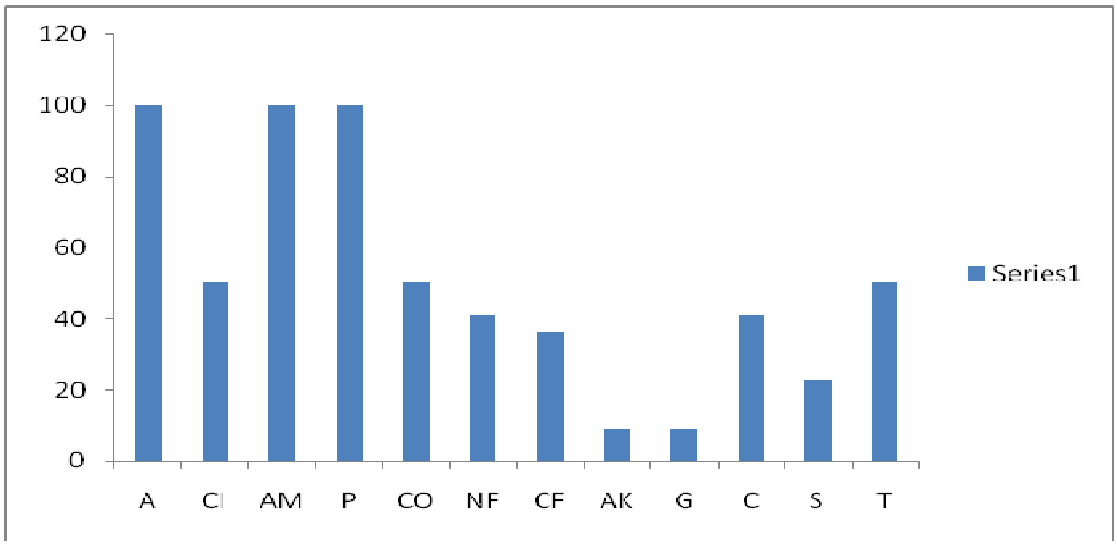

Figure-1
Antibiotic sensitivity of *E. coli*

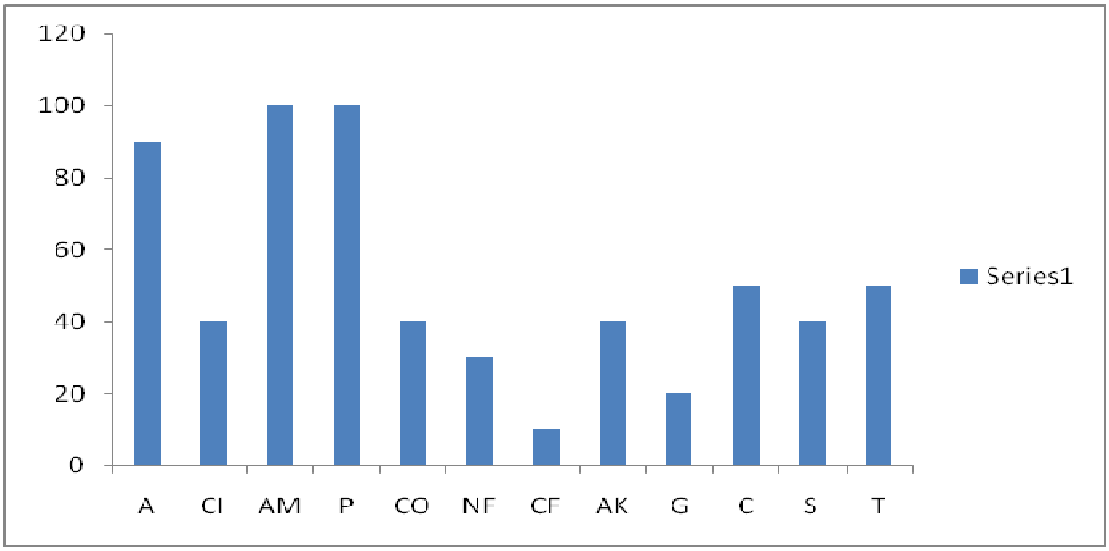

Figure-2
Antibiotic sensitivity of *Pseudomonas aeruginosa*

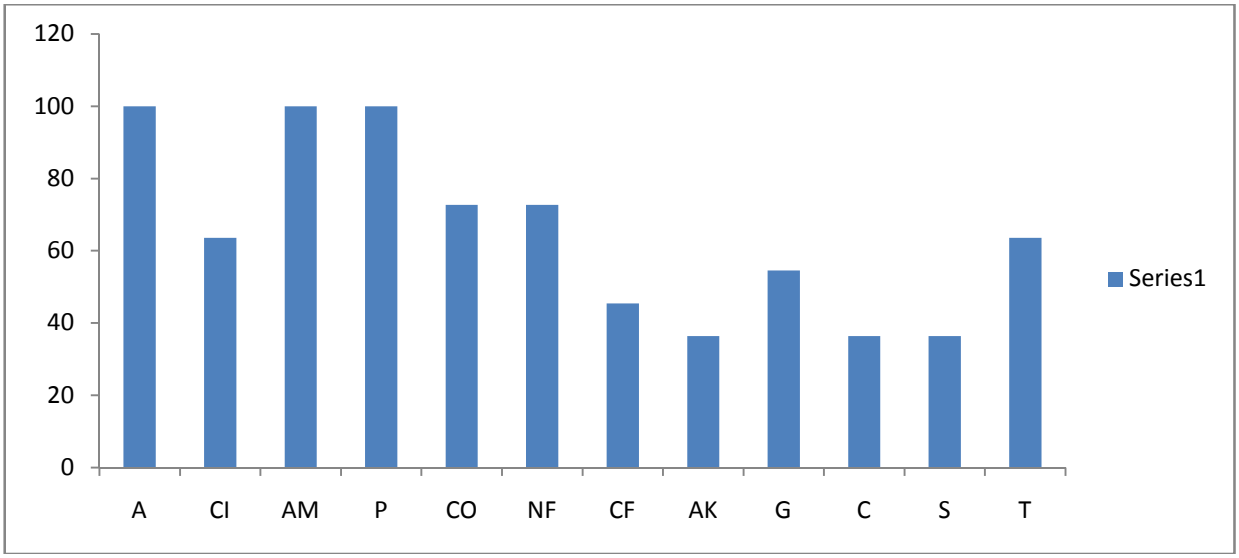

Figure-3
Antibiotic sensitivity of *Klebsiella pneumoniae*

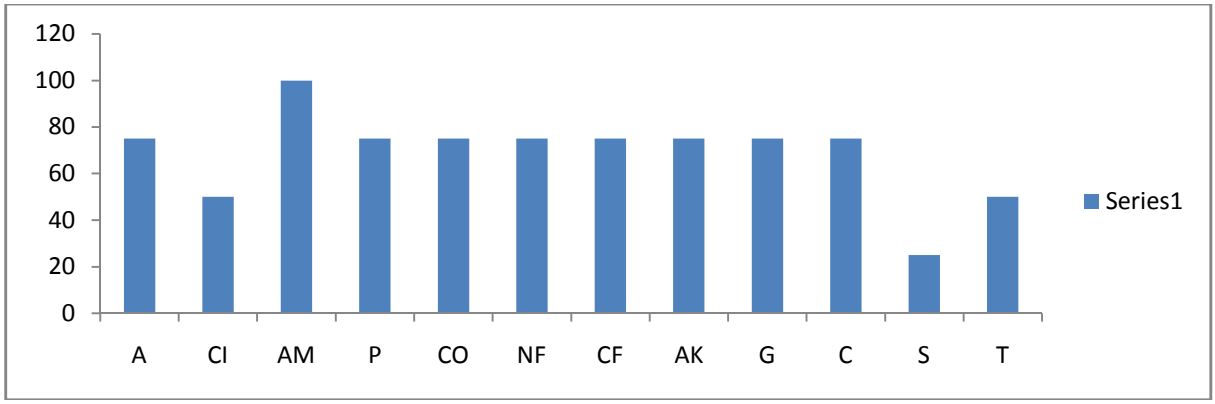

Figure-4
Antibiotic sensitivity of *Proteus mirabilis*

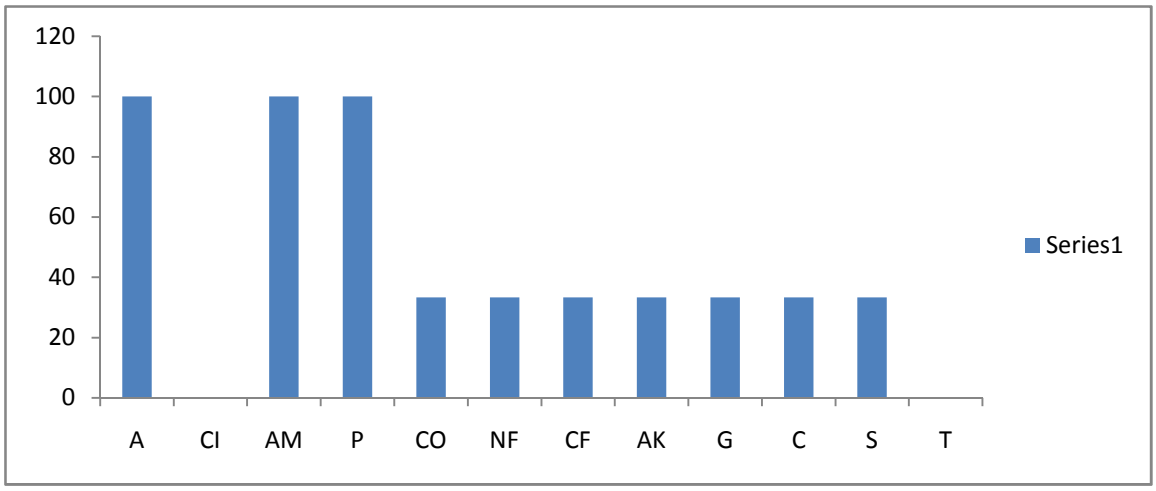

Figure-5
Antibiotic sensitivity of *Staphylococcus aureus*

Table-1
Percentage of Isolates from Clinical Samples

S. No.	Total No. of clinical isolates N=50	Infection	Isolate	Percentage of Isolates
1	22	UTI	*E.coli*	100%
2	12	Catheter	*Klebsiella pneumonia*	100%
3	8	Pus	*Pseudomonas aeruginosa*	100%
4	4	Sputum	*Staphylococcus aureus*	100%
5	4	UTI	*Proteus mirabilis*	100%

Table-2
Morphological Characterization for Clinical Isolates

Organisms	Growth in nutrient agar	Staining	Motility
E.coli	Large thick grayish white moist smooth opaque	Gram negative, Rod	Motile
Staphylococcus aureus	Large circular convex, smooth shiny opaque	Gram positive, cocci	Non-motile
Pseudomonasaeroginosa	Large irregular colonies	Gram negative, Rod	Motile
Proteus mirabilis	Straight rods colonies	Gram negative,Rod	Non-motile
Klebsiella pneumoniae	Large dome shaped	Gram negative, Rod	Non-motile

Table–3
After Plasmid Curing With Pomegranate Peel Extract and SDS

S. No	Name of the antibiotics	*E.coli* (control)	*E.coli* after plasmid curing with pomegranate peel extract	*E.coli* after plasmid curing With SDS
1	Co-trimoxazole	19 mm(S)	20 mm(S)	15mm
2	Netillin	23 mm(S)	26 mm(S)	20mm
3	Amoxycillin	R	R	R
4	Ceftriaxone	6 mm(R)	9 mm(R)	5mm(R)
5	Amikacin	R	R	R
6	Ampicillin	R	R	R
7	Cefamandole	R	R	R
8	Tobramycin	10 mm(R)	12 mm(R)	8mm(R)
9	Streptomycin	R	R	R
10	Penicillin	R	R	R
11	Nitrofurantoin	R	R	R
12	Imipenem	15 mm(I)	16 mm(S)	12mm(R)
13	Ciprofloxacin	R	R	R
14	Gentamicin	13 mm(I)	14 mm(I)	10mm(R)
15	Amoxyclav	R	R	R

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