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PLASMID MEDIATED ANTIBIOTIC RESISTANCE IN *E. COLI* ISOLATED FROM CHRONIC PERIODONTITIS

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ABSTRACT

Background: This study evaluated the antimicrobial resistance and its possible reduction by plasmid of *E. coli* isolated from saliva of chronic periodontitis. **Design and Setting:** *E. coli* sensitivity tests and Plasmid curing was performed in Human Genetics Laboratory, Sree Balaji Dental College and Hospital, India. **Method:** Twenty isolates of *E. coli* from chronic periodontitis were identified using morphological and biochemical characteristics. The antimicrobial sensitivity assay was performed using ceftazidime, ceftriaxone, kanamycin, gentamycin, amoxicillin, ciproflaxacin and ampicillin. Plasmid was identified using antibiotics that were resistant to *E. coli* and its elimination efficiency were further analysed using acridine orange and elevated temperatures. **Result:** The antimicrobial resistance percentage >65% for *E. coli* (n = 20) isolate was identified. Plasmid was distributed randomly up to 3 numbers in 20 different *E. coli* isolates. The plasmid curing efficiency was found to be increased to 94% with the corresponding increase in temperatures up to 45°C. Meanwhile acridine orange at 20 µg/ml and 40 µg/ml showed elimination efficiency of 39% and 67% respectively. **Conclusion:** Decreased resistance to penicillin and cephalosporin group of antibiotics after plasmid elimination might be due to the possible selection of plasmid mediated beta-lactamase producing strains, but less relationship was observed between antibiotic resistance and plasmid in *E. coli* isolates of chronic periodontitis.

KEYWORDS: *Escherichia coli*, Chronic periodontitis,Microbial sensitivity tests, Plasmid.

INTRODUCTION

The rapid spread of multi-drug resistant bacteria means that both common and life-threatening infections have become untreatable and medical interventions made impossible. Oral diseases such as dental caries and periodontal disease are often the reason behind the continuous use of antimicrobials. Antibiotic resistance has become common in the administration of antibiotics alone or in combination with surgery or curettage to treat patients with refractory periodontitis and several types of extraoral infections. The predominance of anaerobic bacteria in periodontitis is mainly due to the initial establishment of common aerobic bacteria.[1] There is always a balance between microbial flora and the human immune system, where this microbial flora acts as a commensal organism with an individual.[2] *Escherichia coli* (*E. coli*) is the most common non-spore-forming gram-negative organism with extended spectrum beta lactamase (ESBL), isolated from cancer patients.[3] The increase in bacterial multidrug resistance recognition rate has also been dramatically increased nowadays.[4] The mobile genetic elements such as plasmids and transposing harbor the antibiotic resistance genes as a marker for resistance development. Plasmids harbor genes coding for specific functions including virulence factors and antibiotic resistance that permit bacteria to survive the hostile environment found in the host and resist treatment.[5] Plasmids in *E. coli* carries different genes such as antibiotic resistance, antibiotic production, virulence genes and sex pilli which facilitates *E. coli* to adopt in adverse condition and allow other bacteria to compete with different environmental situations.[6] Some plasmids undergo spontaneous segregation and deletion, but the majority is extremely stable and requires the use of curing agents or other procedures to increase the frequency of spontaneous segregation. The elimination of plasmid from bacterial isolates establishes the association of plasmid with multidrug resistance.[7] Our present study identifies the antimicrobial resistance percentage, plasmid distribution and its curing ability of multidrug resistant *E. coli* from chronic periodontitis, might reduce the risk factors for infection with multidrug resistance.

METHODS

Isolation of *E. coli*

Chronic periodontitis patients saliva samples (n=20) were recruited from Department of Periodontics,Sree Balaji Dental College and Hospital, Pallikaranai, Chennai, Tamil Nadu, for the study. *E. coli* isolation and morphological identification from saliva of chronic periodontitis (n=20) (Approved from Institutional Ethical Committee Reference No. SBDCH/IEC/09/2016/20) were done using Eosine methylene blue agar (EMB) and was further confirmed biochemically as lactose fermenting, betaglucuronidase and indole positive and oxidase negative.[8]

Antibiotic sensitivity tests

The antimicrobial sensitivity test was performed using disc diffusion method.[9] The antimicrobial agents used were ceftazidime (30µg), ceftriaxone (30 µg), kanamycin (65 µg), gentamycin (50 µg), amoxicillin (10µg), ciproflaxacin (70 µg) and ampicillin (40 µg) (Himedia laboratories). Reference strains of *E.coli* ATCC 25922 was used as control in this study.

Plasmid Isolation and curing

Plasmid DNA isolation was done using various antibiotics including ampicillin, amoxicillin, ceftazidime and ceftriaxone that were resistant to *E. coli* isolate, using standard alkali lysis method.[10] The plasmid curing of the resistant isolate was performed as per standard protocol.[11,12] *E. coli* isolate was matched with 0.5 McFarland standard solution and incubated at 37°C for 18 hours. Plasmid curing was performed with *E. coli* culture in the presence of ampicillin (60 µg) alone. This was inoculated in triplicates containing 20 µg/ml and 40 µg/ml of acridine orange (AO) separately. Elevated incubation temperature (5–7°C) above the optimal growth temperature can be used as a curing method. The isolated resistant plasmids were cured by growing them initially at 39°C. The culture was incubated at further elevated temperature of 42°C until it reaches late log phase, at which time it is diluted (1:20) and re-incubated at 45°Cuntil late log phase growth was attained. A prepared serial dilution was plated to obtain single colonies which were individually tested for loss of the plasmid-encoded trait and physical absence of the plasmid was analysed using agarose gel electrophoresis

RESULTS

Antibiotic sensitivity analysis

The antimicrobial susceptibility pattern of *E. coli* (n = 20) isolate was resistant to amoxicillin (80%), ampicillin (75%), ceftazidime (65%) and ceftriaxone (70%).Meanwhile other antibiotics including kanamycin,gentamycin and ciprofloxacin showed their sensitivity to *E. coli*, and are summarized in Table 1.

Table 1: Antibiotic susceptibility pattern of *E. coli* isolated from saliva of chronic periodontitis.

S. No.	Antibiotics	*E. coli* (n=20)			
		Concentration (µg)	Resistant No (%)	Intermediate No (%)	Sensitive No (%)
1	Ceftazidime	30	13 (65)	3(15)	4(20)
2	Ceftriaxone	30	14(70)	3(15)	3(15)
3	Ampicillin	40	15 (75)	0(0)	5(25)
4	Amoxycilin	10	16 (80)	0(0)	4(20)
5	Gentamycin	50	3 (15)	4(20)	13(65)
6	Kanamycin	65	5 (25)	3(15)	12(60)
7	Ciproflaxacin	70	3(15)	5(25)	12(60)

Plasmid distribution and curing

The distribution of plasmid DNA is shown in Table 2. Plasmid ranged from 1 to 3 in 20 different *E. coli* isolate using ampicillin, amoxicillin, ceftazidime and ceftriaxone that showed resistance to *E. coli* in sensitivity assay. Meanwhile, no plasmid was obtained using other antibiotics, including kanamycin, gentamycin and ciprofloxacin (Data not shown).

Table 2: Plasmid distribution of resistant *E. coli* isolates.

S.No.	Antibiotics used	Number of Isolates	Number of plasmids
1.	Ampicillin	3	3
2.	Amoxicillin	2	3
3.	Ceftazidime	8	1
4.	Ceftriaxone	5	2

Plasmid curing was performed in order to determine the location of drug resistance markers are either plasmid-borne or chromosomal mediated. The elevated growth temperature was used to cure ampicillin-resistant strains of *E. coli*. The plasmid curing by varying temperatures of 39, 42 and 45°C showed the curing efficiency (%) of 31, 78 and 94 respectively. However, these *E. coli* cells did not appear until after several cell generations at the elevated temperatures. Meanwhile acridine orange at 20 µg/ml and 40 µg/ml showed elimination efficiency of

39% and 67% respectively as shown in Figure 1a and 1b.

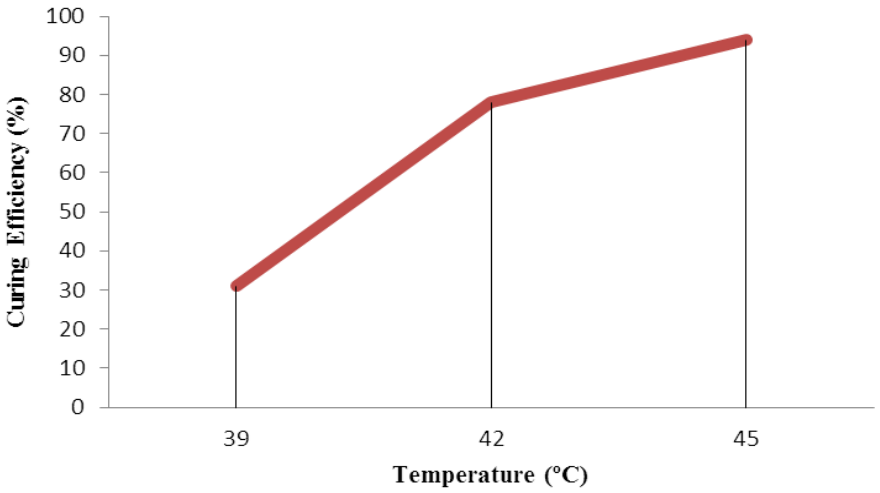

Figure 1a: Graphical representation of plasmid curing by physical method.

Temperatures increase of 39, 42 and 45°C hrs showed the curing efficiency (%) of 31, 78 and 94 respectively.

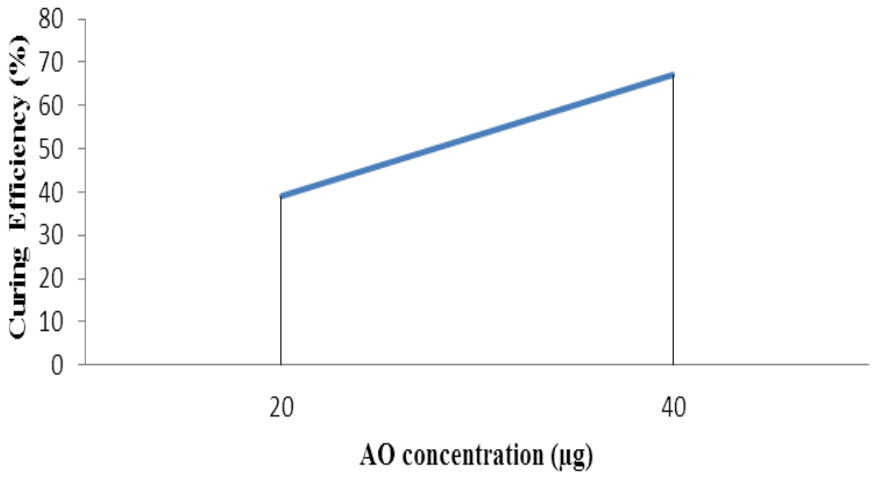

Figure 1b: Graphical representation of plasmid curing by chemical method.

Acridine orange at 20 µg/ml and 40 µg/ml showed the elimination efficiency of 39% and 67% respectively.

Curing was usually observed at a high frequency, and the results obtained were more reproducible with elevated temperatures and acridine orange dye.

DISCUSSION

In the present study, we have analyzed the antimicrobial resistance to *E. coli* isolates from saliva of chronic periodontitis. A minimum of 65% of resistance was observed for penicillin and cephalosporin groups. It is interesting to note that the decreased sensitivity to penicillins and cephalosporins might be due to the selection of beta-lactamase producing strains. The presence of β-lactamases are well known to be the leading cause of antibiotic resistance to β-lactam antibiotics among gram negative bacteria. Numerous reports have confirmed that the plasmid-encoded β-lactamases genes for TEM-1, TEM-2, or SHV-1 by mutations alter the amino acid configuration of these β-lactamases and the most common plasmid-mediated β-lactamase of ampicillin resistant E. coli was TEM-1.[13]

In our results, the plasmid observed in all of the 20 *E. coli* isolates were similar to the report that *E. coli* isolates from chicken were with 81.7% of plasmid prevalence.[14] The plasmid copy number of 5.5 (ranging from 1 – 10) was observed on uropathogenic *E. coli* isolated from children.[15] The slight variation in plasmid number using different antibiotics might be due to the exposure to different antibiotics, bias towards the patient's immunocompromised condition and also due to the occurrence of resistance genes as an evolutionary process. It is conferred that this distribution of plasmid is

in accordance with resistance by the high-level expression of a plasmid-mediated class C β-lactamase as reported.[16] The absence of plasmids in the presence of antibiotics other than beta lactam groups from our data indicated that the antibiotic resistance genes might be located in bacterial chromosomes but not plasmid mediated.

One strategy to minimize plasmid transmission of antibiotic resistance is to eliminate the plasmids. This process is known as 'curing' and many compounds have been shown to be capable of causing this effect. The curing of extracted plasmid from *E. coli* reverts back previously resistant isolates into sensitive form. Plasmid curing efficiency of resistant isolates was found to be increased with the increasing temperature and chemical treatments. Thus the noticeable reduction in curing efficiency using penicillin and cephalosporin group antibiotics might suggest that this could be due to plasmid mediated beta-lactamase producing strains of *E. coli* isolates of chronic periodontitis.

A recent report has shown that plasmid elimination frequency of multidrug resistant *E. coli* was increased upto 11.76% (with 75 µg/ml) for acridine orange.[17] Meanwhile, high curing efficiency (17% to 83%) with 10% SDS was observed in resistant *Pseudomonas aeruginosa*.[18] The ampicillin resistant *Pseudomonas aeruginosa* when treated with elevated temperature exhibited its curing efficiency from 60% to 100%. The effect of elevated temperature on plasmid curing efficiency reverted to wild forms when *Lactobacillus* strains had plasmid for peptidase activity and lactose metabolism.[19]

Some plasmids undergo spontaneous segregation and deletion, but the majority is extremely stable and requires the use of curing agents or other procedures to increase the frequency of spontaneous segregation. Some curing agents work in a non-specific way by damaging and stressing out the cells, while some seem to act much more selectively. The mechanism of plasmid curing starts from the inhibition of plasmid replication resulted from a single nick outside of the replication origin of the super helical form. This process leads to further relaxation of plasmid DNA, an increase in melting point and circular dichroism.[20] Different plasmids vary considerably in their property to be cured and not necessarily depending upon the properties of specific plasmid. There is also a consistent need for on-going antimicrobial resistance surveillance of important and commonly isolated clinically significant pathogens of *E. coli* species which in turn could reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

CONCLUSION

The data of this study concludes the overall reliable identification of antimicrobial sensitivity, plasmid prevalence and its curing efficiency within the limited number of *E. coli*. It needs further detailed analysis of plasmid characterization and determination of plasmid mediated resistance to overcome the problems of multi drug resistance in *E. coli*.

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