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Vinegar - as an Antibiofilm Agent Against the biofilm of UPEC

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Abstract: Background: Biofilm, an extracellular polysaccharide produced by the uropathogenic *E. coli* may responsible for resistant to treatment, recurrent and persistent urinary tract infection. Table vinegars were tried as an antibiofilm agent to eradicate the production of biofilm. Materials & Methods: A total of 212 E. coli isolated from urine samples were quantitatively determined for biofilm production and Apple cider and Grape vinegar were tested for biofilm eradication using microtiter plate assay. Results: Of the 212 *E. coli* isolates, 127 (59.9%) of isolates were capable of producing biofilm, Based on the criteria 86.6% of isolates were weak biofilm producer, 11% were moderate and 3.2% were strong biofilm producers. The apple vinegar prevented biofilm formation in 121(95.3%) *E. coli* biofilm and grape vinegar 120 (94.5%)

1.Introduction:

Microorganisms irreversibly attach to surfaces producing extracellular polysaccharides, resulting in the formation of a Biofilm. The rate of developmental process and regulation of growth depends on cell attachment, number and types of cells in the liquid and its flow rate, intercellular signals, nutrient composition of the medium, antimicrobial drug concentration and ambient temperature also has a role on it(1)

Biofilms are thousand times more resistant to the approaches like physical, chemical, mechanical or antimicrobial removal than planktonic cells. The uses of novel methods are in advancing stageto control biofilms instead of the conventional treatment with antimicrobials and disinfectants. The bacteria capable of producing biofilm, forms a strong relationship with the lining of tissues or organ system, protects themselves from hosts innate factors, prevents the entry of antibiotic thereby guards the bacteria, makes it to persist longer with increased severity of infection. Also Dialysis catheter, urinary catheter associated biofilm plays the major role in recurrent, persistent infection posing to kidney damage and renal failure.

Antibiofilm agent, a matrix targeting agent weakens the polysaccharide by disruption and degradation. Silver nanoparticles, metallic silver, DNAase I cleaves the eDNA in the biofilm matrix, quorum sensing inhibitor etc, are used to prevent the biofilm formation on glass, plastic, titanium surfaces. New approaches using small molecules, enzyme treatments with the ability to weaken the biofilm structure and targets each important phases of biofilm formation are under development and remains to be validated clinically (2).

In ancient time, Vinegar was thought to be useful for treating infections like sores and oral inflammatory conditions. Study report says, Table vinegar, a sour liquid from fermentation of fruits and vegetables have health benefits, prevent and maintain our health. Vinegar composes high acetic acid content act as an antibacterial, antibiofilm agent but corrosive for host tissues. But consumable vinegar with 5% of acetic acid, a trial of using it as an antibiofilm agent serves multiple purpose such as non-corrosive to mucus membranes and epithelial cells, cheaper, easily available etc., providing good nutrients for health(3). In this study, apple cider and grape vinegar were tried as an antibiofilm agent for eradication of biofilm against biofilm producing *Escherichia coli* isolated from urine samples.

2. Materials and Methods:

Bacterial isolates:

Urine samples obtained from the suspected UTI patients were cultured and the isolated *E. coli* bacteria were confirmed using standard culture methods - 5% sheep blood agar, Macconkey agar, Hicrome UTI agar and biochemical methods. A total of 212 *E. coli* isolates were subjected for quantitative biofilm detection.

Microtitre Plate assay for biofilm production – Quantitative method:

3 to 5 *E. coli* colonies were suspended in 5 ml of Trypticase soy broth (TSB) and incubated for 24 hrs at 37°C. The turbid broth was diluted and adjusted to 0.5 McFarland turbidity standards to reach 10^5 CFU/ml. A 200 µL of diluted bacterial suspension was added to each well of 96-well flat-bottomed polystyrene microtitre plates with 0.25% glucose and incubated for 24 hrs at 37°C. Media with suspended bacteria were then removed; the plates washed 3-4 times with PBS carefully, air-dried and was fixed using 99% methanol, then stained with 200 µL of 0.9% crystal violet (1),(4), (5). After washing the dye, the attached bacteria solubilized with 95% ethanol and the optical density of the adherent biofilm was determined twice with a filter of 450/630nm in ELISA reader (Biorad).

As a negative control, 200 µL of TSB broth with 0.25% glucose were used as a negative control to obtain a background absorbance, which was then deducted from absorbance values obtained from the wells containing study isolates. All isolates were tested in triplicate. The interpretation of biofilm production done based on the criteria, depending on standard calculations (6) the study isolates were classified as the following:

Table: 1

Biofilm production	Biofilm criteria
Non-producer	OD450/630 ≤ ODcontrol
weak	ODcontrol< OD450/630 ≤ 2ODcontrol
moderate	2ODcontrol < OD450/630 ≤ 4ODcontrol
strong	OD450/630 > 4ODcontrol

An optical density (OD) of 0.0601 was chosen as guideline to distinguish biofilm producers from those that did not form biofilm.

Antibiofilm activity of Vinegar against *E. coli* biofilm - in vitro:

Two different types of vinegar – Apple cider and Grape vinegar were used, it has the component especially acetic acid, which has been used as disinfectant against various contaminants (7), and also have antimicrobial potential (3). Distilled water was used as a negative control to show inability of biofilm removal comparing to vinegar against the isolates.

Determination of bactericidal activity of vinegar against biofilm was performed by using modified microtiter plate method (8) (9) (10) with some modifications. This assay was made by 10µL (1:10) of the raw material of vinegar and each type was added to prewashed biofilm of standardized bacterial suspension a in microtiter plate for each study isolate, with gentle agitation and incubated for 18 hrs at 37°C. The contents of each well was aspirated and washed three times with 250 µL of PBS. Plate was shaken well so that non-adherent bacteria were removed. The bacteria attached to the walls were then fixed and washed. The wells were stained with crystal violet and re-solubilized as the previous way in quantitative determination of biofilm (11). The optical density (OD) of each well was measured at the same previous wavelengths by ELISA reader. Similarly the optical density reading interpretations was depended on the biofilm criteria.

Results:

Biofilm formation by *E. coli* from urinary isolates:

Of the 212 *E. coli* isolates, 127 (59.9%) of isolates were capable of producing extracellular polysaccharide (biofilm)

Table: 2 Formation of Biofilm in *E. coli* – Microtitre plate Assay

Bacterial isolates (no.)	Biofilm formation		
	Strong	Moderate	Weak
E. coli (127)	4 (3.2%)	14 (11%)	110 (86.6%)

Table: 3 Antibiofilm effects of Apple cider and Grape vinegar on biofilm producing *E. coli*

Bacterial Isolates (no.)	Biofilm after addition of Apple cider vinegar			Biofilm after addition of Grape vinegar		
	Strong	Moderate	Weak	Strong	Moderate	Weak
E. coli (127)	4 (3.2%)	2 (1.6%)	0%	4 (3.2%)	3 (2.4%)	0%

Growing bacterial isolates in vitro on polystyrene plates in trypticase soy broth supplemented with apple cider vinegar, grape vinegar at (5% acidity). After 24 hours of incubation time, the wells were stained with CV and OD value 450/630nm was measured. The results is shown in table (2) and table (3).The apple vinegar prevented biofilm formation in 121(95.3%) *E. coli* biofilm producer except 6 isolates which was interpreted based on the readings. In grape vinegar 120 (94.5%) isolates were eradicated, except 7 isolates. In 1 moderate biofilm isolate, apple cider vinegar eradicated the biofilm but not by grape and also in two isolates the biofilm eradicated by grape but not by apple cider vinegar.

Discussion:

E. coli is one major bacteria causing urinary tract infection. Biofilm is one of the causes for recurrent, persistent UTI infection, emergence of antibiotic resistance and development of multidrug resistance of *E. coli* leads to dysfunction, damage of kidney and renal failure.

Biofilm formation among uropathogenic *E. coli* is determined by quantitative microtitre plate assay. Out of 212 *E. coli* isolates, 127 (59.9%) of isolates were capable of producing biofilm. Based on the criteria 86.6% of isolates were weak biofilm producer, 11% were moderate and 3.2% were strong biofilm producers.

Classification of bacteria as weak, moderate, or strong biofilm producer is regulated by diverse factors, including the adhesion molecules, host environment, bacterial resistance and the growth medium (10). Weak positive may express the bacteria may be under stress condition or the growth is weak that made the biofilm weak or cannot be produced. These different responses of bacteria to environmental conditions could be the results of mutations in genes that control biofilm formation (12), a strong dependence on sugar supplementation as we add 0.25% of glucose in TSB which is essential for biofilm formation and its growth condition (2). This might be the reason that diabetic mellitus subjects are more to UTI especially *E. coli* and the recurrence of UTI in the subjects may be due to biofilm formed by the bacteria as it is supplemented with high glucose level, a factor which induces the biofilm production

Crystal violet microtitre plate assay is a simple and rapid quantitative method, a standard technique for rapidly accessing cell attachment and biofilm formation in a range of gram positive and gram-negative bacteria as well as yeast comparing to safranin. It is a basic dye known to bind to

negatively charged molecules on the cell surface as well as polysaccharides and nucleic acid and therefore gives on overall measure of the whole biofilm (13).

Vinegars has a clear inhibitory effect on the formation of the biofilms; this result is in agreement with results obtained by [14] in *Streptococcus pyogenes* study. All of them considered as table vinegar due to the limitation of their acidity not more than 5% in order not to be harmful for living tissues when used by human. The activity of acetic acid in prevention of biofilm formation may be attributed to the depression of the intracellular pH by ionization of the dissociated acid molecule, direct pH reduction of the substrate, or disruption of substrate transport by alteration of the biofilm bacterial permeability (15).

Drinking apple cider vinegar (ACV) daily increases acidic environment in urinary tract and discourages the growth of UTI causing bacteria. ACV impairs cell integrity, organelles and protein expression (16). It has a natural antibiotic effect - absence in expression of DNA starvation protein, citrate synthase, isocitrate and malate dehydrogenases in *E. coli*; Grape vinegar (GV), It possesses potent components that decrease the growth of pathogenic organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella species* in urine. The extract is shown to destroy pathogenic bacteria within 15 minutes of contact with a diluted solution (17) (18).

In this study, as in table 3 - both the table vinegars (ACV, GV) completely eradicated the weakly formed biofilm but in case of strong and moderate biofilm the Optical density values were low in post washed biofilm of vinegar added wells compared to the control proves that the vinegar's action in reducing the rate of development of biofilm. Though not significant, one isolate which produced biofilm was eradicated by apple cider but not by grape vinegar, this may be due to high phenolic compound of ACV and other component variation in the vinegars.

There were no observable difference among types of vinegar; apple vinegar is essential role in biofilm eradication. This effect may be due to high content of phenolic compounds in apple the direct relationship between phenolic content of apple extract and the antimicrobial effect on human pathogens (17) (18). Grape vinegar has the extract of high levels of polyphenols which inhibits biofilm. It has been found to include flavanol, polyphenols, anthocyanins and catechins, which exhibited different antimicrobial activity, strong bactericidal and is used in traditional medicine to cure infections (19) (20).

Conclusion:

Vinegar has both antimicrobial as well as antibiofilm effect. Moreover, apple cider and grape vinegar might reduce the colonization of bacteria and are low in calories; their consumption is associated with a number of health benefits. It is widely available; affordable; and as a remedy and adjunct therapy for individuals has yet to be determined.

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