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Variation of Antibiotic Sensitivity of *E. coli* Strain against Norfloxacin Among Two Persons

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Purpose of this experiment was to determine *E. coli* strain variability between two people. Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is due to the use of antibiotics as growth promoters in foods of animals. A study published in the journal in Aug 2007 found that the rate of adaptive mutation in *E.coli* "on the order of 10^{-5} per genome per generation which is 1,000 times as high as previous estimates" a finding which may have significance for the study of bacterial antibiotic resistance. The author became interested in this idea from the similar previous projects viewed and relatives in the prescription medicine field. The project rose the interest further when the authors found that bacterial immunity to antibiotics was on the rise, and becoming a very important issue.

Key words: Biofilms, Recalicrant, Enterobacteria.

E. coli is a gram-ve rod-shaped bacterium that is commonly found in the lower intestine of warm blooded organism. *E.coli* are not always confined to lower intestine, they are also found outside the body, which makes them ideal indicator organism to test environmental sample for contamination (Thomps, 2007). *E. coli* is now introduced to enterobacteriaceae family gamma proteabacteria (Kubitschek, 1990). *E. coli* also causes numerous types of illnesses. SHIGA toxin producing *E.coli*, have also been transmitted by flies (Heuvenlink *et al.* 2002); Uropathogenic *E.coli* is responsible for urinary infections; which results in the infection of the urinary tract to the bladder as well as to the kidneys (De.Boer & Heuvenlink 2000). Biofilms –producing *E. coli* recalicrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infection. *K-antigen* producing *E. coli* infection are commonly found in the upper urinary tract (Johnson *et al*., 2006).

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MATERIALS AND METHODS

Urine sample were taken from two different person's, one regularly using antibiotics (sample 1) and other rarely using antibiotics (sample 2) were taken and directly sprayed over Nutrient agar media (yeast extract-0.25gm, peptone-0.50gm, NaCl-0.50gm, agar-1g, 100ml distilled water) contained in Petri plates. The plates were incubated at 12-24 hrs at 37°C, white colonies were observed. These colonies were inoculated in Nutrient broth (Beef extract-0.15gm,peptone-0.50gm, NaCl-0.50gm,100ml d.w.) mentioned sample1 and sample2. After then loop full of suspension from the broth sample 1 and sample 2 were spread over Macconkey agar media (Peptone 2 gm, lactose 1 gm, bile salt 0.50gm,

NaCl 0.50gm, neutral red 0.009gm, agar 1gm, d.w100ml) ; pink colonies were observed after 24 hour to 48 hour of incubation. Pure culture of *E.coli* is obtained by streaking the inoculums on Eosine-Methylene blue medium (peptone1gm, Dipotassiumhydrogensulphide 0.20mg, lactose 0.50gm, sucrose 0.50gm, Eosin-y-0.40gm, methylene blue 0.0065gm, agar 1gm, d.w 100ml) which shows presence of green metallic sheath.

Various test were done to show both the samples are *E.coli*.Microscopic observation of both the samples was done by performing gram staining technique. Motility determination test of each isolates was performed by hanging drop slide preparation.

Identification of *E. coli* by biochemical test:starch hydrolysis test was performed in media (starch 0.20gm, peptone 0.50gm, beef extract 0.30gm, agar 1.5gm, distilled water 100ml)

Nitrate reduction test was performed by inoculating both samples in nitrate broth (potassium nitrate 0.10gm, peptone 0.50gm, beef extact 0.30gm, d.water 100ml).

IMVIC test

Methyl Red and Voges Proskauer test was performed by inoculating the sample in a MRVP broth (potassium hydrogen phosphate 0.50gm, glucose 0.50gm, tryptone 0.50gm, distilled water 100ml). Indole production test was done by inoculating the sample in tryptone broth (tryptone 1gm, calcim chloride 0.10gm, Nacl 0.50gm, distilled water 100ml).

Citrate utilisation test was processed by streaking both the samples in Simmon's citrate media ($MgSO_4$ 0.02 gm, Ammonium dihydrogen phosphate 0.10gm,Dipotassium phosphate 0.10gm, Sodium Chloride 0.50gm, Bromophenol blue 0.008 gm, agar 1.50mg, distilled water 100ml)

Antibiotic sensitivity test of *E. coli* against norfloxacin

To determined antibiotic sensitivity against *E.coli* test are done by spreading Bacterium suspension over the agar plates. Then small, circular, sterile disc(made from whatman filter paper No.1) saturated with antibiotic (Norfloxacin) are placed on the bacterium covered agar plates, the plates are incubated. After incubation zones sizes around is disc are recorded for resistance or not.

RESULTS

From this project it is investigated strain isolated from the urine samples are *E. coli* by using the differential media Macconkey agar media (Peptone 2 gm, lactose 1 gm, bile salt 0.50gm, NaCl 0.50gm, neutral red0.009gm, agar 1gm, d.w100ml) and Eosine-Methylene blue medium (peptone 1gm, Dipotassium hydrogen sulphide 0.20gm, lactose 0.50gm, sucrose0.50gm,eosin-y0.40gm,methylene blue0.0065mg,agar 1gm,d.w 100ml). For the confirmation of *E. coli* strain biochemical strains test were performed during starch test *E. coli* shows white shining, when the pates were floats' on gram's iodine, it indicates both the samples can hydrolyze starch, and in Nitrate reduction test both samples developed blue colour on addition of nitrate reagent and dil. sulfuric acid. During Indole production of *E.coli* development of ring indicates bacterial samples were *E. coli* When MRVP test were performed, it is observed that methyl red indicator remains red which confirmed that isolated samples were *E. coli*. During VP test there is no change in the colours of the samples, Indicates negative VP which indicates both the samples were *E.coli.* The confirmatory citrate utilization test of *E.coli* shows no change in colours, Hence *E.coli* is citrate negative. After isolation and confirmation of *E.coli* from urine samples,Antibiotic sensivity test of both samples were performed against Norfloxacin. In the antibiotic sensivity test sample 1 colonies existed in the zone of inhibition of Norfloxacin. These colonies of *E.coli* have develop some resistant property.

Colony morphology of samples on agar plate

Sample	Size(mm)	Shape	Elevation	Edge	Color	Cell morphology
1	2	circular	flate	entire	cream	bacillus
2	2	circular	flate	entire	cream	bacillus

Physical test of samples

Sample	Gram Reaction	Cell Size(µm)	Oxygen use	Glucose Use	Endospore	Motility
1	Negative	2	Faculative	Yes, gas	no	yes
2	negative	2	Faculative	Yes, gas	no	yes

Biochemical test of both sample

Sample	Starch Hydrolysis Test	Nitrate Reduction Test	Indol Production Test	Methyl Red Test	Voges proskauer test	Citrate Utilization Test
1	positive	positive	positive	positive	negative	negative
2	positive	positive	positive	positive	negative	negative

Antibiotic sensitivity test of sample I against norfloxacin

Sample	Number of times	Antibiotic	Dilution	Zone of inhibition
1	1	Norfloxacin	5mg/ml	10mm
1	2	Norfloxacin	7 mg/ml	12.5mm
1	3	Norfloxacin	9 mg/ml	14mm

Antibiotic sensitivity test of sample II against norfloxacin

Sample	Number of times	Antibiotic	Dilution	Zone of inhibition
2	1	Norfloxacin	5 mg/ml	23.5mm
2	2	Norfloxacin	7 mg/ml	24mm
2	3	Norfloxacin	9 mg/ml	26mm

DISCUSSION

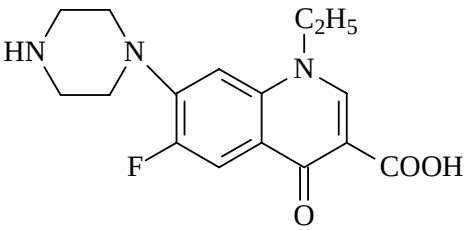
Sample1 colonies exhibited resistant against Norfloxacin.

Reason 1

Norfloxacin has invitro against broad range of gram negative bacteria.The fluorine atom in Nor floxacin at the 6 position provides increased potency against gram negative organisms. Norfloxacin inhibits bacterial deoxy ribonucleic acid synthesis and is bacteriocidal at the molecular level, 3 specific events are attributed to norfloxacin in *E.coli* cell.

1. Inhibition of ATP-dependent DNA supercoiling reaction catalyzed by DNA gyrase.
2. Inhibition of the relaxation of supercoiled DNA.
3. Promotion of double- stranded DNA breakage.

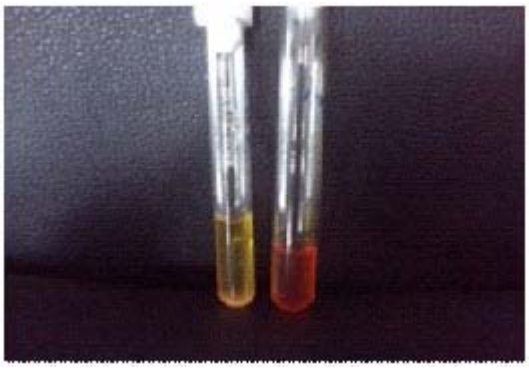
As the sample 1 is taken from the person regularly using antibiotics i.e., Norfloxacin as a result *E.coli* adopt mutation in its genome. Such as resistant to norfloxacin.



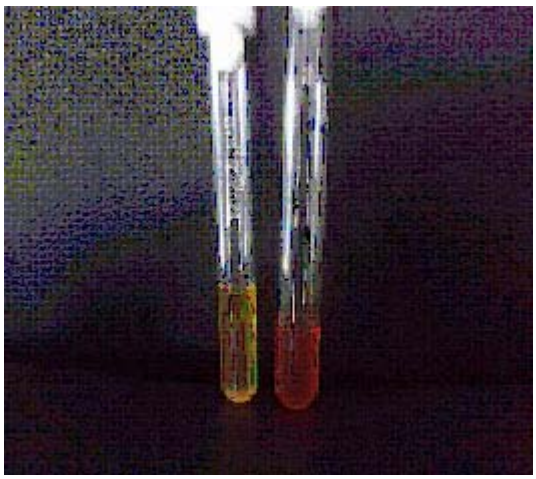
Norfloxacin

Reason 2

E.coli often carry multi drug resistant plasmids and under stress readily transfer these

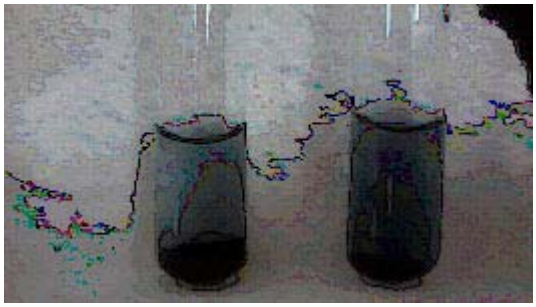


Methyl Red Test Sample I

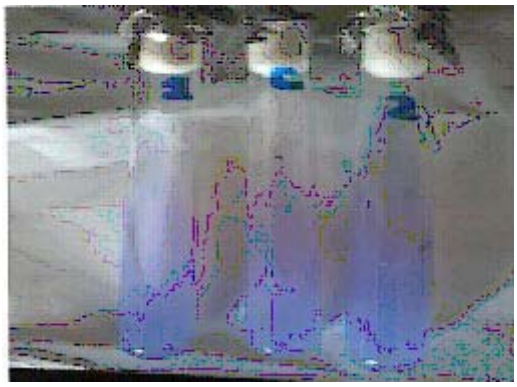


Methyl Red Test Sample II

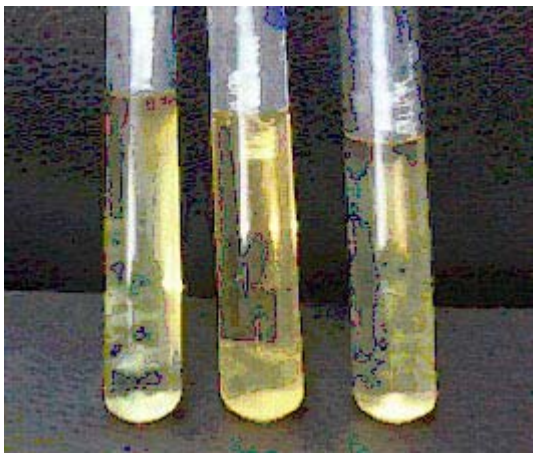
Indole Production Test Sample I, II



Nitrate Production Test Sample I, II

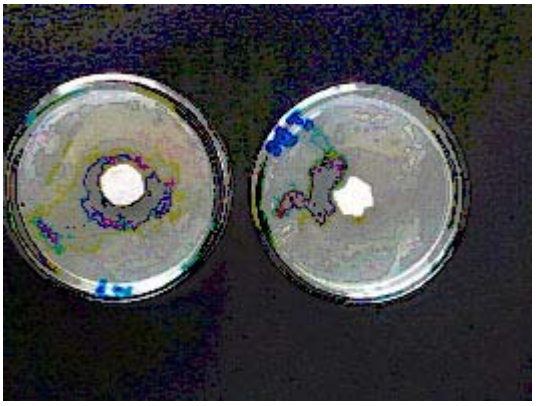


Citrate Utilisation Test Sample I,II

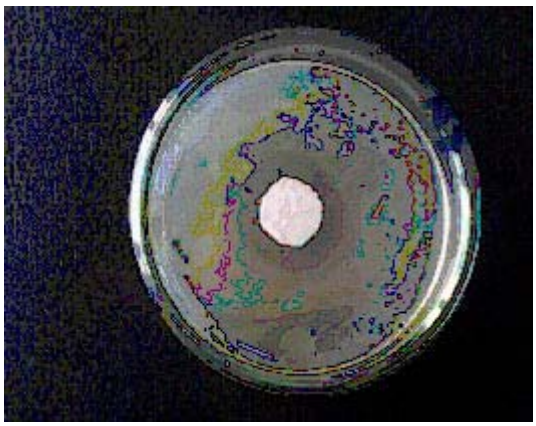


Voges Proskauer Test Sample I, II

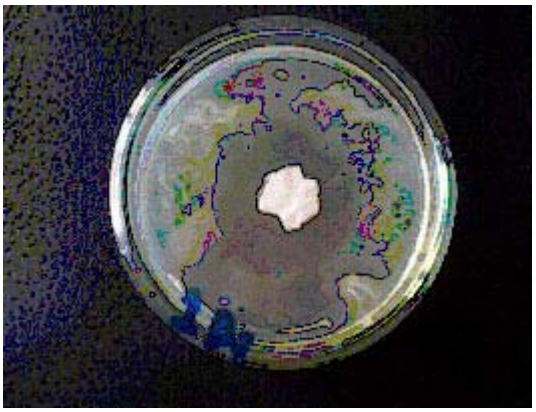
Starch Hydrolysis Test Sample I, II



Antibiotic Sensitivity Test Sample I, II



Antibiotic Sensitivity Test Sample1



Antibiotic Sensitivity Test Sample II, Against Norfloxacin

Plamids to other species. Indeed *E.coli* is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E.coli* strains that are piliated to accept and transfer plasmids from one to another bacteria. Thus, *E.coli* and other Enterobacteria are important reservoirs of transferable antibiotic resistance.

CONCLUSION

Mutation and natural selection are driving forces of evolution, only leads to loss of functional system. Therefore antibiotic resistance of bacteria is not an example of evolution in action but rather a variation in bacterial kind. If mutant strain changes out, further research can be carried out but it depend upon environmental condition.

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