

EFFECT OF SUBMINIMAL INHIBITORY CONCENTRATION OF GENTAMICIN ON THE PATHOGENICITY OF ESCHERICHIA COLI CAUSING INFANTILE DIARRHOEA

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ABSTRACT: One hundred patient infant complaining from infantile diarrhoea were studied, the percentage of *E. coli* infection was 24%. The in vitro adherence test was done to determine the adhesion capacity of bacteria to Human Buccal Epithelial Cells (HBECs). The result shows, increasing in the mean adhesion of *E. coli* as compared with the control group. Minimal Inhibitory Concentration (MIC) and sub Minimal Inhibitory Concentration (subMIC) of gentamicin was done to study the effect of subMIC of this antibiotic on the adhesion , the result shows that the using of gentamicin in lower doses (subMIC) decreases the adhesion capacity of bacteria .EPEC still mediclly important as a danger pathogen especially in poorly hyagiene communities such as Iraqi community.

Key words: E. coli, infantile diarrhoea, gentamicin

INTRODUCTION

Gentamicin antibiotic inhibits protein synthesis of bacteria by attaching to and inhibiting the function of the 30S subunit of the bacterial ribosome, its activity increased at alkaline pH. Gentamicin has been used in serious infections caused by gram negative bacteria insusceptible to other drugs (Jawetz et. al, 1998)

The Enteropathogenic *Escherichia coli* (EPEC) had been associated with infantile diarrhoea (Sheldon, 1987). *E. coli* is the most commonly recognized bacterial pathogen in infantile gastroenteritis (Khan et. al, 1988).

Pili or fimbriae are non flagellar filamentous surface appendages of some bacteria especially intestinal commensal and pathogenic species of the enterobacteriaceae family (Jawetz et. al, 1998). Pili are very important virulence factor of *E. coli* that mediate adherence to the intestinal mucosa and promote infection (Low et. al, 1984).

The adhesion of EPEC is a two-stage mechanisms: (1) an initial attachment of bacteria to the intestinal mucosa and (2) effacing of microvilli and intimate EPEC attachment, which may occur in the absence of the first

stage (Embaye et. al, 1989). The majority of fimbriated bacteria bear fimbriae of a type that enable them to adhere to the surface of the cells of many animals and human (Tomoyuki et. al, 1983).

METHODS

1- Specimen collection

Stool: stool specimens were collected from each patient.

Rectal swabs: rectal swabs were collected from 10 healthy infants to isolate *E. coli* bacteria as control group.

2- patients:

One hundred patients were included in the present study which were admitted to pediatrics hospital in Ramadi. The age of infants ranging from few days – 2 years

3- Human Buccal Epithelial Cells (HBECs)

Cells were collected by scraping the oral mucosal membranes of a healthy person with wooden applicator and were suspended in phosphate buffer saline (PBS), then the cells were centrifuged at 3000 rpm washed 3 times and resuspended in PBS at a concentration of 2-10 cell / ml as determined by haemocytometer, then the cells were used in the adhesion test with the test bacteria (Sugarman and Danta, 1979).

4- Cultivation of stool.

This method was used to isolate *E. coli* bacteria from the specimen; this was carried out directly within one hour of collection into MacConkey and blood agar plates and was incubated at 37°C for 24 hours.

5-Identification of EPEC

This was achieved by morphological characters of the colonies on MacConkey agar , by biochemical tests by IMViC (Baily and Scott, 1986)

and by serodiagnosis of *E. coli* with *E. coli* specific antisera 1 and 2 Polyvalent antisera (bioMerieux) (Al-Fahdawi, 2002).

6- Preservation of *E. coli*

As used by WHO procedures by using the glycerol method (10%) (Vandepitte et. al, 1991).

7- In vitro adherence test

One ml of overnight culture of *E. coli* suspension was gently added to one ml of buccal epithelial cells suspension. The mixture was mixed and then incubated at 37°C for one hour.

To get rid of unattached bacteria to the HBECs, these cells were washed four times with PBS.

A drop of final HBECs suspension was put on the glass slide, air dried, heat fixed, and gram stained, and then the slide was examined by direct light microscope under oil immersion (Tomoyuki et. al, 1983).

8- Determination of MIC:

As experiments of Baily and Scott, 1986.

RESULTS

Figure 1 shows the incidence of *E. coli* in acute infantile diarrhoea. From this result, it is obvious that the percentage of *E. coli* infection is 24% while 76% of other causes.

Figure 2 shows the mean adhesion of *E. coli* to HBECs pre and post treated with subMIC of gentamicin as compared with the control group. It is obvious that the adhesion capacity of *E. coli* was reduced after treated with subMIC of gentamicin in approximately 50% as compared with pretreated isolates.

Results of MIC and subMIC of gentamicin for five *E. coli* isolates were: MIC ranging (1.25-2.5) and subMIC (0.312-1.25) microgram /ml.

DISCUSSION

The diarrhoea continues to be a major infectious disease of infants all over the world.

The present study involved 100 infants suffering from diarrhoea. EPEC predominates 24% of the total etiology. This result agrees with Katouli et. al, 1990 in Iran who reported that 26.7% of the infantile diarrhoea cases were due to *E. coli* but disagrees with Khan et. al, 1988 in Pakistan who reported that 43% of infantile diarrhoea was due to *E. coli*, this difference may be related to the difference in economic and sanitary levels in these countries.

The ability of bacteria to adhere to the receptors of the epithelial cells is very necessary step in the pathogenesis of the bacteria (Al-Fahdawi, 2002. goldhar et al, 1987), the ability of *E. coli* isolates to adhere to the HBECs was demonstrated in figure 2. the mean adhesion of bacteria to HBECs was significantly differ from the adhesion pattern of non pathogenic *E. coli* (control), from this result it is obvious that most bacterial strains express or have the genetic potential to express the adhesins. The isolates of the control group showed decrease in the number of attached bacteria (poorly attached) that may be due to the bacteria express the mannose sensitive adhesins but the pathogenic *E. coli* (EPEC) with high potential (highly attached) due to the mannose resistant adhesins(Al-Fahdawi 2002). A possible explanation for this observation could be differences in the size of HBECs, cell age, and a day to day variation of bacteria is an important factor (Kallenius et. al, 1980).

Several studies showed that the use of gentamicin antibiotic might lead to impairment of an in vitro bacterial adherence if these antibiotics are present in subminimal inhibitory concentrations during bacterial growth (Al-Ubaidy, 1990). It is obvious that from the present study that the gentamicin antibiotic in a concentration of 0.312 mcg/ml was useful to decrease the adhesion capacity of *E. coli* to HBECs, this by using the lower dose than the MIC of certain antibacterial agent can alter some surface structures of the bacterial cells so as to prevent its adherence to human epithelial cells without killing it this may be due to inhibition of synthesis of the bacterial structures responsible for adherence possibly the pili. The use of gentamicin antibiotic lead to inhibition the protein synthesis and causes inhibition of pili mediated adhesion probably by direct inhibition of the synthesis of the fimbrial protein itself, together with inhibition of a (helper) polypeptide synthesis which is probably involved in the secretion of the pilus-protein, in addition to that the bacterial enzymes which are involved in the protein synthesis may be affected (Chopra & Hacker, 1986). The results of the present work are in agreement with Al-Ubaidy 1990 who reported that the treatment of *E. coli* with antibacterial agent will remove the fimbriae of bacteria thus prevent the adhesion and pathogenesis of bacteria.

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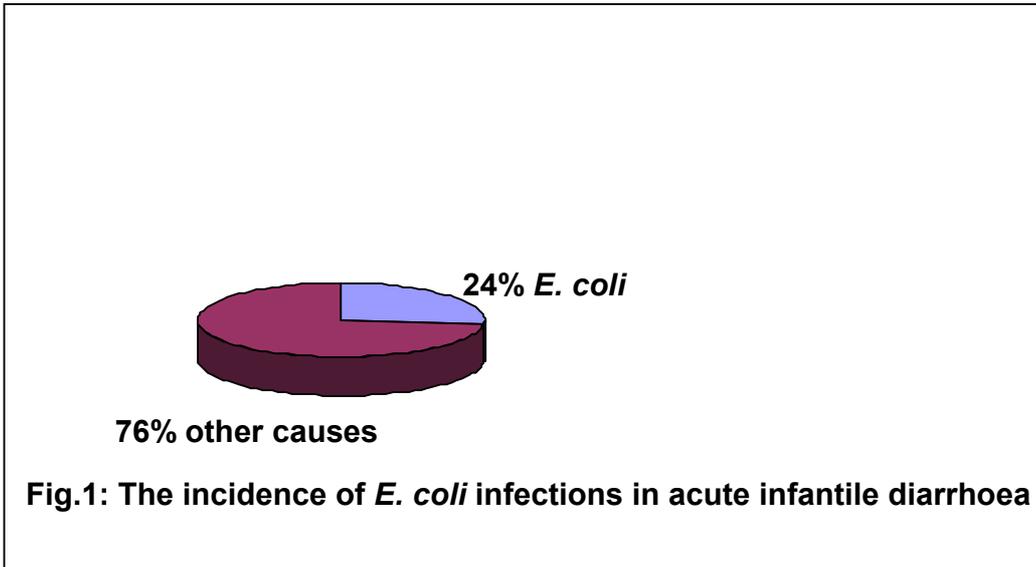


Fig.1: The incidence of *E. coli* infections in acute infantile diarrhoea

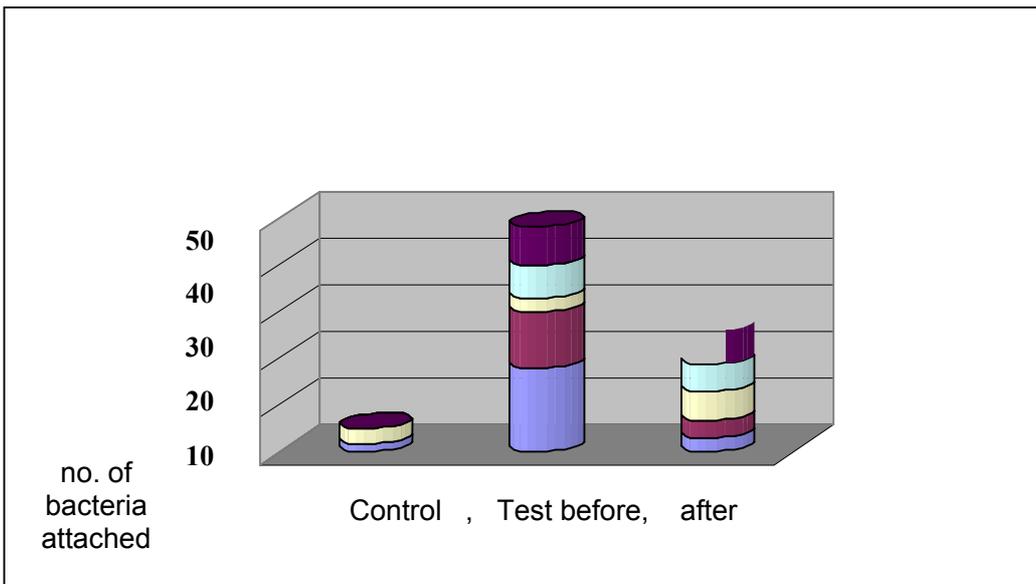


fig.2: The mean adhesion of *E. coli* to HBECs pre and post-treated with subMIC of gentamicin compared with the control

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