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Antibiotics 2020: How far is the effect of sub minimal inhibitory concentration (Sub MIC) on virulence factors expressed by bacteria? - Nida'a M.A. Wadi - National University of Science and Technology

Nida'a M.A. Wadi
National University of Science and Technology, Oman

Statement of the Problem:
The major virulence factors associated with infections are the ability of microorganism to adhere to the tissue and initiate the interaction of bacterial cell with epithelial cells to cause infection. Antibiotic medications are widely used in the treatment and prevention of various infections. Sub inhibitory concentrations (Sub MIC) antibiotics may produce antibacterial activity (Jahanshahi et al., 2010). It may have an effect on bacterial structure, influence the adhesion of bacterial to epithelial cells, and prevent colonization. Sub MIC of antibiotics can exert their anti-virulent effects in different ways in such way to interfere with the ability of the microorganisms to approach the receptors on cell surfaces of host tissue and innate immunity system able to improve cell response to infection (Ofek *et al.*, 1079; Dong *et al*, 2019).

The Sub MIC effects lead to the development of a new antimicrobial potentiating drug aimed to prevent the infection and an alternative approaches to the treatment of drug-resistant bacteria by anti-virulence approaches. The SubMIC of Antibiotics ampicillin, gentamicin, cephalothin and carbenicillin can effect on adhesion virulence factors of uropathogenic *Escherichia coli.* (Emody, *et al.*, 2003; Wojnicz *et al.*, 2007; Shah *et al.*, 2019)

Methodology and Theoretical Orientation:
Urine samples were collected from patients reported as UTIs. Mid-stream urine from 600 patients suffering from UTIs with age, 17-70 years was examined. Identification and antimicrobial susceptibility test were conducted on all isolates.

Examination and cultivation of urine
Urine sample was cultured on selective media like MacConkeey agar after 18-24hrs.the agar examined for colony of bacteria. The colony represent bacterium from the original sample and thus called colony –forming unit (CFU) to estimate number of bacteria per milliliter of urine.

Biochemical test
Biochemical test were carried out indole test, voges- proskaur test, methyl red test and simmon's citrate agar test. When E.coli shows the above reactions as per guideline the different E. coli isolates were inoculated onto agar slant and stored at refrigerator (Versteeg et al., 1985; Finegold et al., 1986).

Adhesion test
The adhesion test for the *E.coli* was applied by method of slide hemagglutination of erythrocyte cells (Iwahi et al., 1983). Bacterial cells from individual colonies was picked up by sterile loop and mixed with one drop of normal saline on microscope slide to form homogenous emulsion. One drop of 4% human RBCs group suspension was added and mixed by wooden applicator for 1 min. The hemagglutination was then observed either macroscopically or by microscope within one minute. The test was aided by two controls bacterial suspension and normal saline. The second one was RBC's suspension and normal saline to observe any auto agglutination or clumping (Duguid & Old, 1980; Dong et al, 2019).

Bacterial strains and susceptibility testing
One hundred and one E.coli isolates with hemagglutination positive were collected from different patients with urinary tract infection. Antimicrobial susceptibility testing was conducted on all isolates using disc diffusion method (Bauer-kirby).

Prior to performing antibacterial test, turbidity of all bacteria was standardized to 0.5McFarland standard (Hindler, et al., 1990). A single colony from pure culture was isolated and inoculated in fresh broth media. Inoculums size was standardized by comparing with 0.5McFarland standard prepared freshly before antibacterial test. Plates of Luria – Bertani (LB) were then inoculated by immersing sterile cotton swabs in diluted cultures and passing them over the agar surface. Fourteen different antibacterial agents were used. A control strain of E.coli ATCC25922 (Difco) was used with each batch .Temperature of incubation 37ºC, the zone of inhibition diameter was taken at 18-24 hr.

Determination of MIC and sub MIC
In order to demonstrate the concentration of antibacterial drug that show inhibition of bacteria quantitatively, tube dilution method was applied. This gives a more accurate estimation to the degree of sensitivity of microorganism to the drug. Five different antibiotics were used Ampicillin 500mg/vial, gentamicin 80mg/2cc, carbenicillin 1gram/vial and cephalothin 1 gram/vial. Serial two fold dilutions of the above antibiotics were prepared. Briefly, fourteen clinical isolates with hemagglutination positive were grown overnight in Luria – Bertani (LB) broth were prepare. Representative was chosen to examine the MIC and SubMIC. E.coli ATCC25922 (Difco) served as the control strain. Positive control, which contains bacteria and broth while negative control, contains diluents and broth and the third control contains antibiotic solution with broth.

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All tubes were incubated for 18-24hrs.at 37ºC to observe the presence and absent of turbidity (Finegold & Martin, 1986).

Findings:

The results showed 363(82.5%) had Uropathogenic *E.coli* and 215 (59.2%) they showed hemagglutination positive. The susceptibility of *E.coli* to different antibiotics showed that nitrofurantoin, nalidixic acid, gentamycin, rifampin were more effective in comparison tetracycline, sulfonamides.

For the determination of the lowest concentration of an antibiotic that inhibits the growth of E.coli (MIC), Ampicillin inhibits the growth of 14 hemagglutination E.coli strains except one strain. This is demonstrated when a drop of bacterial culture from the Sub MIC tubes were tested with erythrocytes, no hemagglutination was seen. They can showed range from ½ MIC to ¼ MIC. Similarly, for gentamicin all isolates loss their adherence virulence factor character with negative hemagglutination when treated with ½ or ¼ MIC, while only three showed positive hemagglutination. All the 9 isolates showed negative hemagglutination reaction when they pretreated by ½ MIC or ¼ MIC of carbenicilin. The remain E.coli isolates were resistant to all the drug concentrations used. Similarly the antibiotic sensitivity tube method test was run for the 14 E.coli isolates and the result showed eight isolates showed negative hemagglutination test when they pretreated with ½ or ¼ MIC of cephalothin drug. This explains that the bacteria become unable to adhere to the epithelial cell. While six isolate retained it virulent factor and showed positive hemagglutination.

Conclusion and Significance:

The result demonstrated that Subminimum inhibitory concentration of certain antibiotics can alter some surface structure sufficiently to prevent bacterial adhesion of Escherichia coli strains and subsequently reduced the colonization. This gives more chance to the defense mechanism in the urine to be sufficient to prevent bacterial adhesion.

Investigating the effects of Sub MIC antibiotics bacterial adhesion to epithelial cells may lead to the development of future antibiotic treatment modalities and may suggest a new parameter for the use and the study of antibacterial agents. Sub MICs of antibiotics can potentially influence the outcome of infection by altering bacterial virulence factor expression and by- pass antimicrobial resistance. Alternative chemicals and natural product at sub MIC may offer the opportunity to explore more about new strategy to overcome bacterial resistant.

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