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Mecillinam and Fosfomycin Susceptibility in multi-drug-resistant *Escherichia coli* Isolated from Urine Samples.

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ABSTRACT

Uropathogenic *E. coli* (UPEC) is the major cause of over 90% of urinary tract infections (UTIs). UTIs are the major common bacterial contaminations in health facilities and society settings. These infections require immediate medical attention as they cause discomfort to the diseased people. The cure of this disorder is complicated with the arrival of extended-spectrum β-lactamase (ESBL)-manufacturing pathogen. Therefore, the objective of this study is to determine the impact of two ancient antibiotics: Fosfomycin and Mecillinam. The experiment was conducted on diverse multidrug resilient clinical isolates. The challenges associated with the contaminations are compounded by the lack of appropriate chemotherapeutic agents. Several trials were conducted to investigate the vulnerability of clinical isolates like AmpC, + ESBL, TEME, and OXA 48. *E.Coli* is grown under intracellular and extracellular conditions of two longstanding antibiotics, namely Mecillinam and Fosfomycin. The antibiotics are viewed as disruptive against a range of Gram-negative bacteria. Our outcome demonstrates that these antibiotics show a lethal effect on some bacteria under study. Lastly, this investigation endorses the probable usage of both Mecillinam and Fosfomycin in the cure of these multidrug resistant microorganisms.

Keywords: Pathogen, antibiotics, ESBL positive strains, and *E.coli*

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INTRODUCTION

A urinary tract infection is caused by upper urinary tract-kidney contaminations and lower urinary tract infection-Bladder toxicities. The impact on the urinary tract is a common occurrence in females. It is usually caused by a main casual factor known as *Escherichia coli*. Bacteria, fungi, or viruses can also cause it. Antibiotics can be used to cure UTIs; however, resistance to several antibiotics is rising. Some of the symptoms associated with this problem include urinating and burning sensation [1,2,3,4,5]. Another sign is the absence of vaginal discharge. The bacteria that cause this infection normally reach the bladder via the urethra. However, it can also be through blood or lymph. Transmission of bacteria to the urethra through the bowel occurs frequently. Females are at a higher risk because of their anatomy. Afterward, it enters the bladder after attaching itself to the bladder wall. It later develops a biofilm that repels the body's immune reaction. The urinary tract structure comprises of the bladder, urethra, kidneys, and ureters. The main purpose of the kidney is to filter out the body's urine [6,7,8,9].

Urinary tract infection (UTI) happens when different pathogens contaminate any of the organs of the urinary zone. Infections of the urinary tract are classified into three types, namely the urethritis, pyelonephritis, and cystitis. Urethritis affects the urethra, while cystitis is a contamination of the bladder. Finally, pyelonephritis affects the kidneys. The bladder infection is the easiest to treat among the three. Acute simple urinary infection happens when it presents as severe cystitis. Non-obstructive pyelonephritis is a common occurrence among people with acute cystitis [9,10].

Asymptomatic chemotherapeutic bacterial contamination occurs when there is a presence of transmittable bacteria in a urine specimen. At this stage, no signs of the urinary area infection are seen. This infection can be recurring because its origin is harbored within the urinary tract. People with anomalies in the genitourinary tract are prone to complex urinary tract infections. Research indicates that cystoceles, higher volume of residual urine, and bladder diverticulitis result in the reappearance of UTIs. Additionally, urinary tract contamination is a function of genetic tendency and advanced frequency of deformities in genitourinary organs. UTIs commonly affect the old people in society. This is a result of the use of indwelling devices such as catheters. Some of the clinical manifestations of this disorder comprise of urgency to urinate, irritation, fever, and bladder infection among others [12,13,14].

Prevalence of UTI in Global Epidemiology:

The world-wide epidemiology study assessments of ESBL production among *E. coli* differ globally. The smallest proportion of ESBL manufacture in *E. coli* was discovered in Latin America. Other cases were witnessed in Asia, Europe, and North America. Estimates indicate

that occurrence of ESBL-producing pressures of *K. pneumonia* and *E. coli* is low in America. In contrast, it is higher in Asia-Pacific region than in other zones of the world such as Latin America. The percentage of putative ESBL- manufacturing isolates can differ according to zones [15,16,17].

A research that was conducted in the US medical centers indicated that 6%–7% of Klebsiella isolates attained the standards for a possible ESBL- generating phenotype. More than half of these isolates displayed resistance to clavulanate-inhibitable cephalosporin. This was after confirmatory investigations on ESBL phenotype were conducted. Almost 2,000 *K. pneumoniae* isolates and *E. coli* from the Asia-Pacific area produce a reasonably bigger percentage of potential ESBL. Isolates are showed as ESBL phenotype on screening among North Americans. 43% were confirmed as ESBL makers [18]. On the other hand, 180% of the probable ESBL producers were identified in Western Pacific and Latin America. These outcomes demonstrate that the predictive value of screening for ESBL phenotype is uppermost in areas where ESBL-manufacturing creatures are predominant [19,20,21].

According to Ansari 2018, it was evident that in Spain, CTX-M-positive stresses were more resistant to diverse classes of antibiotics than bacteria manufacturing other kinds of ESBLs. Nevertheless, it was discovered that strains that produced -14 or CTX-M-9 were more resilient to ciprofloxacin and tetracycline than those that produce SHV-12 or TEM-4. The CTX-M genes are associated with genetic structures like sul1-type. It might describe the multidrug nature of creatures manufacturing these enzymes. In addition, cassettes assist in the resistance of aminoglycosides and b-lactams. Inadequate information is available about the molecular epidemiology of CTX-M- producing microorganisms from the public. Moreover, readings from the UK and Spain showed that majority of *E. coli* manufacturing CTX-M enzymes from the society had no clonal association. However, investigations in the UK indicated that there was hereditary association among strains manufacturing CTX-M-15 [22].

Extended spectrum beta lactamase (ESBL): classification and properties:

Beta-lactamases enzymes are formed through specific microorganisms by which resistance to beta-lactam antibiotics like penicillin's and carbapenems is provided. They are adequately resilient to beta-lactamase [23]. One of the ways that is commonly used to express *Enterobacteriaceae* is through plasmid-encoded β-lactamases. Other categories of enzymes known as extended-spectrum b-lactamases were discovered in Germany in the mid-1980s. ESBLs are used to hydrolyze extended-spectrum cephalosporins. This is usually done through the help of an oxyimino side chain. Most often, beta-lactamase in Gram-negative microorganisms is TEM-1creatuion. This results in around 90% of ampicillin resilient in *E. coli*. It causes penicillin and ampicillin resistance seen in *H. influenza* and *N. gonorrhoeae*. Substrates such as ceftriaxone and cefepime are different from oxyimino-beta-lactam. CTX-

M beta-lactamases are recognized for their activities against cefotaxime [24,25,26]. Oxacillin and associated anti-staphylococcal penicillin might be hydrolyzed via OXA beta-lactamases. Research shows that they have hydrolytic activity against cloxacillin and oxacillin. Additionally, they have scanty clavulanic acid and provide resistance to ampicillin and cephalothin. On the other hand, ESBL phenotype can be given through amino acid replacements in OXA enzymes. Majority of antibiotics are seen in *E. coli*, *Enterobacteriaceae*, and *K. pneumoniae*. There is normally a separation of AmpC type β-lactamases from extended-spectrum cephalosporin-resilient gram-harmful microorganisms. Some examples of these bacteria comprise of Citrobacter, Enterobacter, and Serratia species. C/group 1 is also known as AmpC β-lactamases. It would be on *Escherichia coli* and be hyperexpressed, though not normally inducible. It may hydrolyze broad and lengthy-spectrum cephalosporins. However, they are not inhibited by clavulanic acid, which belongs to β-lactamase category of inhibitors [27,28,29].

Bacteria belong to the family of Enterobacteriaceae. They include *Klebsiella pneumoniae*, which create extended-spectrum beta-lactamase. They are usually more hazardous compared to the multi-resilient *Staphylococcus aureus*. ESBL pathogens are dangerous to the lives of individuals as they can cause bile duct illnesses. ESBL can spread quickly if individuals fail to observe simple hygienic practices. This happens if someone who is infected touches another individual with unwashed hands. Besides, it can spread if one touches surfaces that have microorganisms. Healthcare workers are advised to wash their hands when they are exposed to diseased people [30,31,32].

These contaminations should be controlled since they can result in loss of lives. Investigations reveal that ESBL producing *E. coli* is the leading cause of deaths globally. It is estimated that it causes more than 60% of these deaths. However, it is possible to decrease these problems by sensitizing the public about the disorder [33,34,35].

The Treatments:

Antibiotics were initially categorized as natural drugs produced by fungi or bacteria and differed from other chemotherapeutic drugs but with the advent of synthetic antibiotics, this categorization has been abolished [36]. Moreover, new drugs have been developed from the naturally occurring antibiotics by binding various side chains to the basic structure. The term antibiotic was introduced by S.A. Waksman in the year 1942 almost fourteen years after the discovery of the first antibiotic, Penicillin by Alexander Fleming. Penicillin was effective against most of the dangerous pathogens of that time including pneumococci, streptococci, staphylococci, meningococci, gonococci, Corynebacterium diphteriae and Treponema pallidum. Streptomycin was another very important discovery as it killed most Gram negative pathogens as well as Mycobacterium tuberculosis. However, with the advent of the

phenomenon of antibiotic resistance most of the antibiotics became non-effective against the resistant strains. This problem was temporarily solved with the extensive discovery of new antibiotics but many of them like neomycin and colimycin proved to have high toxicity and there usage had to be limited. Discovery of semi-synthetic antibiotics like methicillin and ampicillin in the 1960s did provide some relief against Gram negative bacteria like *E. coli*, H. influenza, and S. enteric. Irrational use of antibiotics and the development of resistant strains like bacteria producing ESBLs and AmpC made antibiotics like cephalosporin's useless against the ESBLs producing Enterobacteriaceae.

Carbapenems can be used against such ESBL producing Enterobacteriaceae and can be used to treat UTIs.

Carbapnems are the broadest spectrum β lactams and are effective against both Gram positive as well as Gram negative organisms. They are mostly used as antibiotics of last resort to treat very critical patients or patients suffering from infections by multidrug resistance pathogens.

Carbapenems being β lactam can still evade inhibition by most β lactamase (serine lactamases.) as these behave as "slow substrates". Most class A β lactamases are inhibited by clavulanic acid but most class D and none class C β lactamases are not inhibited by clavulanic acid. However, all class A, C and some class D β lactamases are inhibited by carbapenems. [36,37,38]

Among the carbapenems, imipenems are very effective in the treatment of UTIs caused by ESBL producing Enterobacteriaceae.It is commonly known as primaxin and is derived from a compound called thienamycin, produced by Streptomyces cattleya. Imipenem is however susceptible to degradation by the enzyme dehydropeptidase-1 (DHP-1) and so requires administration with a DHP-1 inhibitor such as Cilastatin. The discoveryof carbapenems stable to degradation by DHP-1 like merpenem, ertapenem and doripenem avoids the need of a DHP-1 inhibitor.Imipenem has an in vivo half-life of approximately 1 hour.Apart from being administered for the treatment of UTIs, Imipemen is also used in the treatment of nosocomial pneumonia, meningitis, intra-abdominal infections and febrile neutropenia

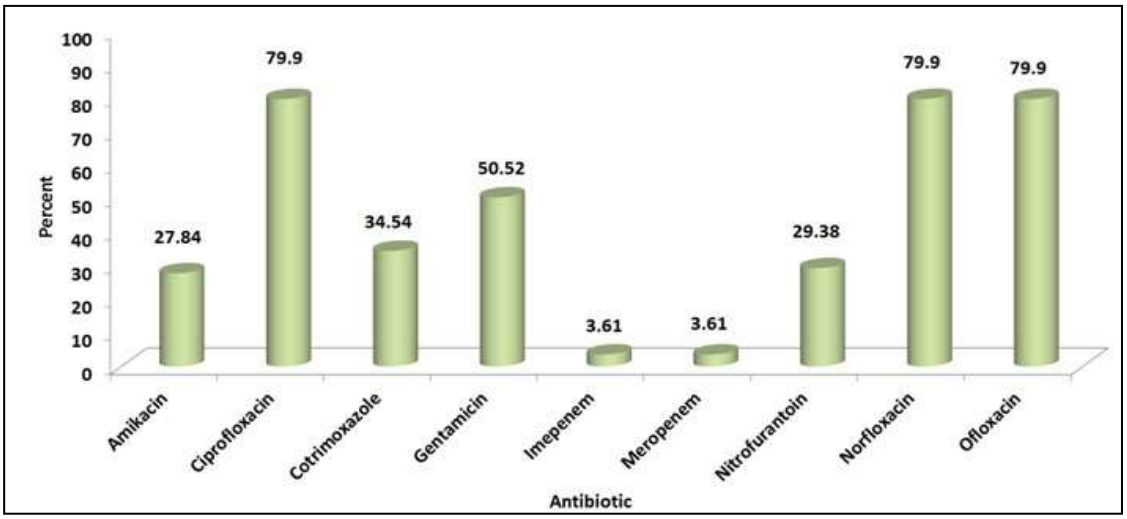

Figure 1: The Pattern of resistance to various antibiotics of Enterobacteriaceae causing UTIs.

However, it has been reported that certain Enterobacteriaceae unpredictably may acquire carbapenemase activity. In order to treat such cases antibiotics like Mecillinam and Fosfomycin may be used. .Mecillinam may not be effective against very serious infections of ESBL producing *E. coli* [39,40,41,42].

Mecillinam:

Mecillinam is an extended-spectrum penicillin antibiotic that binds particularly to penicillin binding protein 2 (PBP2) and it is only regarded as active against Gram-negative bacteria. It is used mainly in treating bladder infections, and has additionally been accustomed to treat typhoid and paratyphoid fever [43].

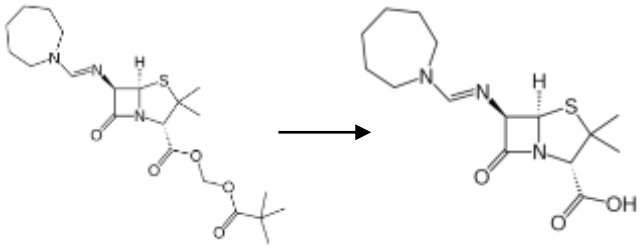

Figure 2: The Structure of Pivmecillinam (left), which is hydrolysed to Mecillinam (right) in vivo. (www.wikepedia.org)

For the reason that Mecillinam has really low dental bioavailability, an orally active prodrug was created: pivmecillinam. Neither drug will come in the U. S. By means of the code name FL 1060, Mecillinam was created through the Danish pharmaceutical company Leo Pharmaceutical Items . It was initially referred to within the scientific literature inside a 1972 paper.

Worldwide potential to deal with Mecillinam in bacteria leading to UTIs has continued to be really low since its introduction a 2003 study carried out in 16 European nations and Canada

found potential to deal with vary from 1.2% *Escherichia coli* to five.2% *Proteus mirabilis*. Another large study carried out in Europe and South America acquired similar results - 95.9% of *E. coli* strains, for example, were responsive to Mecillinam [44,45,46,47].

Fosfomycin:

Fosfomycin which is a phosphonic acid derivative may also be used to treat uncomplicated UTIs caused by E. coli. It is available in the form of Fosfomycin trometamol, which is soluble salt with disodium and can be used intravenously. Fosfomycin is broad-spectrum antibiotic discovered inside a joint effort of Merck and Co. and Spain's company Española de Penicillin y Antibiotics (CEPA). It was initially isolated by screening broth cultures of *Streptomyces fradiae* isolated from soil samples for the opportunity to cause formation of spheroplasts by growing bacteria. The invention was referred to in a number of papers released in 1969. CEPA started creating Fosfomycin with an industrial scale in 1971 at its Aranjuez facility.

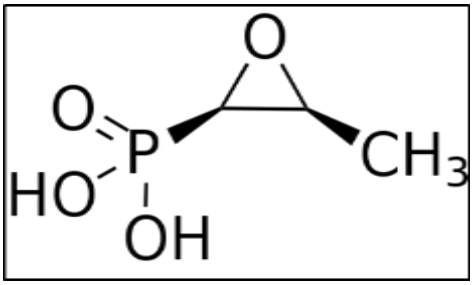

Figure 3: The Structure molecule of Fosfomycin salt. (www.wikepedia.org)

Fosfomycin is suggested for treating bladder infections, where it is almost always given like a single dental mega-dose. Its use in conjunction with tobramycin to deal with lung infections in patients with cystic fibrosis seemed to be investigated.

The medication is well tolerated and it has a minimal incidence of dangerous side effects. However, growth and development of microbial resistance under treatments are a regular occurrence and makes Fosfomycin unacceptable for sustained therapy of severe infections.

Additional uses happen to be suggested. The worldwide problem of evolving antimicrobial resistance has brought to some restored curiosity about its use more lately .Moreover, Fosfomycin resistant ESBL producing *E. coli* are rare. Thus, Fosfomycin may be used to treat UTIs caused by ESBL producing *E. coli* [48,49,50,51,52].

CONCLUSION:

UTIs are some of the infections that commonly affect people. One of the risks is that they can interfere with the renal system. Individuals are advised to go for diagnosis whenever they see any signs of urinary tract infection. The goal is to know one's status before commencing treatment. However, those who do not have the disorder should try to prevent it. One of the ways is by maintaining better hygienic practices. Research indicates that UTI infection is

prevalent among the kids. This is because they cannot maintain proper hygiene without the assistance of their guardians or parents. The disorder is harmful as it can lead to complications in the renal system. Therefore, it is essential to go for a diagnosis before commencing treatment. Those who may not have the disorder should uphold their condition by observing better hygiene. Moreover, it is essential to manage this condition to avoid recurrences in the future. Renal ultrasound is one of the best approaches to diagnosing some fundamental congenital abnormalities. These circumstances have the potential of enhancing recurrence risks. As a result, one may require a surgical operation to rectify the condition. Various forms of medications such as Fosfomycin can be used to treat the disorder. These drugs are commonly used to heal bladder infections. Other types of cure include the use of antibiotics that help to eliminate harmful pathogens in the human body. Mecillinam is another drug that has been used to reduce bacterial infections since 2003. Additionally, those who provide primary healthcare should wear protective devices.

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