DOI: 10.14260/jemds/2014/3666

ORIGINAL ARTICLE

QUINOLONE RESISTANT E. COLI - A SILENT INVADER IN ELDERLY PATIENTS: A STUDY FROM EASTERN INDIA

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HOW TO CITE THIS ARTICLE:
Rajdeep Saha, Anirban Bhaduri, Paramita Das, Kalidas Rit, Arnab Mallik, Papiya Biswas, Jaya Das, Dipanwita Pal. "Quinolone Resistant E. Coli - A Silent Invader in Elderly Patients: A Study from Eastern India". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 55, October 23; Page: 12577-12583, DOI: 10.14260/jemds/2014/3666

ABSTRACT: BACKGROUND: Quinolone resistant Escherichia coli associated urinary tract infection in geriatric age group poses a very serious problem. **AIMS:** To find out the prevalence of quinolone resistant Escherichia coli causing geriatric urinary tract infection and their electropherotype determination by agarose gel electrophoresis. **MATERIALS AND METHODS:** The hospital based prospective study included elderly patients aged 65 years and above who were admitted, or visited the outpatient departments in the hospital, and had confirmed UTI. **RESULTS:** Escherichia coli (69.6%) were most common offending agent isolated from urine. Among them, 73% was quinolone resistant. Isolated quinolone resistant Escherichia coli were further sub typed by agarose gel electrophoresis to know their pattern of resistance. **CONCLUSIONS:** Fluroquinolone resistant Escherichia coli have emerged as a potential threat causing both communities acquired and nosocomial urinary tract infection and monitoring of resistance is necessary to prevent treatment failure and increased morbidity and mortality with UTI.

INTRODUCTION: Urinary tract infection (UTI) is the second most common infectious complaint in geriatric age group overall.[1] The diagnosis and treatment of UTI in the elderly is much more complicated than adults. Though Escherichia coli ranks first both as causative agent of nosocomial and community acquired UTI, The fluoroquinolones (FQs) are potent antimicrobial agents used for the treatment and prophylaxis of infections caused by gram-negative bacteria, including E. coli. There is not so much information about the recent status of quinolone resistant E. coli in elderly patient suffering from UTI in our hospital. This study was an effort to find out the incidence of quinolone resistant E. coli infection among geriatric group of population and to know their type of resistance pattern through electropherotyping in our tertiary care hospital of eastern India.

MATERIALS & METHODS: This prospective study was done at our tertiary care hospital from September 2013 to July 2014. The study included all geriatric patients (65-96 yrs) who were admitted or came in outpatient departments in hospital with symptoms of UTI during the study period and had UTI confirmed by positive urine culture reports. Subjects with clinical symptoms of UTI but no growth and commensal growth on culture were excluded from this study. Subjects who were treated with quinolone antibiotics within the previous 48 hours were also excluded from the study.

Overall, 1006 subjects were included in the study (male: 628, female: 378). A clean catch midstream urine specimen, or suprapubic aspirate in subjects who were unable to give the former, was collected in a sterile, wide-mouth, leak-proof container. Using a calibrated loop method of loop

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diameter 4 mm, 0.001 ml of uncentrifuged urine was transferred onto the Mac Conkey agar plate using standard loop technique and incubated at 35- 37^{0}C for 24 hours. A specimen was considered positive for UTI if a single organism was cultured at a concentration of >10^5 colony forming units/ml. The Gram-positive and Gram-negative organisms' culture isolates were further identified by using various biochemical reactions up to genus/species level.

In the presence of any potential growth of E.coli, antibiotic sensitivity testing was done by the Modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[2] The antibiotics tested were Imepenem, Meropenem, Ciprofloxacin, Norfloxacin, Ofloxacin, Amikacin, Gentamicin, and Cotrimoxazole (High-Media, Mumbai).

Fluroquinolone resistance pattern in E.coli was further confirmed by agarose gel electrophoresis. At first DNA was isolated from 300 samples by phenol chloroform extraction and ethanol precipitation method by following steps:

Step 1: Disruption of the cell membrane by lysozyme and release of the DNA into a medium containing saline solution and EDTA in which it was soluble and protected from degradation.

Step 2: Dissociation of the protein-DNA complexes by detergents such as Sodium dodecyl sulphate (SDS) which were used to disrupt ionic interaction between positively charged histones and negatively charged backbone of DNA.

Step 3: Separation of the DNA from other soluble cellular components.

Before precipitation of DNA, deproteinization of solution was brought about by treatment with chloroform/isoamyl alcohol and followed by centrifugation. Upon centrifugation, three layers were produced- i) upper aqueous phase ii) a lower organic layer, and iii) compact band of denatured protein at the interface between the aqueous and organic phases.

The upper aqueous phase containing nucleic acids was then separated and the DNA precipitated by addition of ethanol. The DNA formed a threadlike precipitate that can be collected by "spooling" onto a glass rod. The isolated DNA was contaminated with protein and RNA. Protein was removed by dissolving the spooled DNA in saline medium and repeating the chloroform/isoamyl alcohol treatment until no more denatured protein collected at the interface.

RNA was degraded during the procedure by treatment with ribonuclease after the first or second deproteinization steps. After isolation, the DNA molecules were electrophorosed by agarose gel electrophoresis method and visualized under ultra violet light after staining with an appropriate dye (Ethidium bromide).

Agarose gel electrophoresis was performed by following Steps:

Step 1: Preparation of Agarose Gel: Agarose gel was prepared by mixing 1 gram of Agarose to 100 ml of buffer and mixture was swirled to suspend the Agarose powder in the buffer. After placing the gel solution into the microwave oven, solution was boiled and swirled until all of the small translucent Agarose particles were dissolved. Then the molten Agarose was cooled to 60 °C.

Step 2: Casting Agarose Gel: Agarose gel was cast with UV-transparent plastic (UVTP) tray directly on the stage of the Sub-Cell GT bases using the gel casting gates.

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Step 3: After the agarose gel was solidified, sample was loaded and electrophoresis was started.

Step 4: After completion of electrophoresis, the molecules in the gel were stained with Ethidium bromide and the gel was placed on a UV transilluminator for nucleic acid visualization and analysis. Simultaneously plasmid analysis was done to detect resistance pattern of E.coli against quinolone.

RESULTS AND ANALYSIS: Out of 1006 urine samples 228 (22.6%, n=1006) showed growth of no bacteria and in 142 samples (14.1%, n=1006) there were growth of commensal. Out of rest 636 urine samples, Escherichia coli (442, 69.6%, n=636) was most common offending agent isolated from urine followed by Klebsiella sp (10%, n=636), Staphylococcus aureus (7.9%, n=636), Pseudomonas aeruginosa (6.9%, n=636), Citrobacter koseri (0.9%, n=636), Serrattia marcescens (0.7%, n=636), Proteus mirabilis (2.2%, n=636) Acinetobacter baumannii (1.8%, n=636).

Diagram 1: Showing distribution of isolated bacteria causing UTI in geriatric age group.

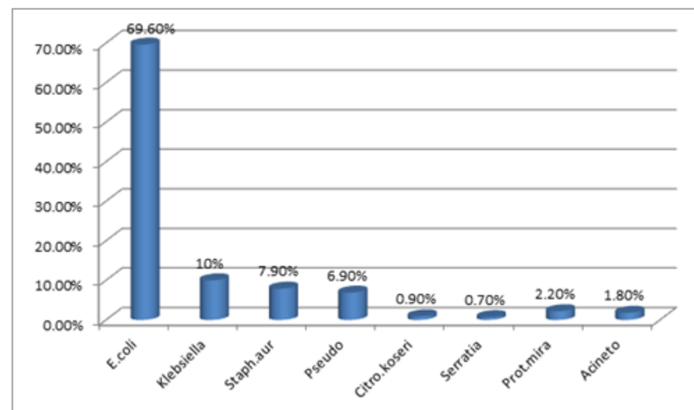

Diagram 1

Out of 442 Escherichia coli isolates 323 (73%) showed resistance to quinolone antibiotics.

Diagram 2: Showing distribution of quinolone resistance among isolated Escherichia coli.

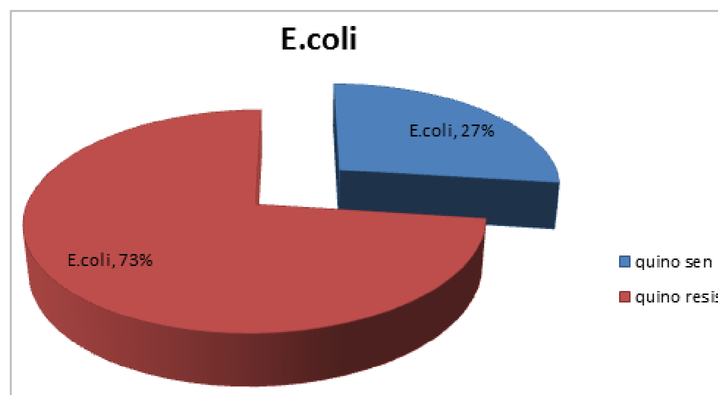

Diagram 2

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Out of 323 quinolone resistant E. coli isolates, 300 isolates were electrophorosed by agarose gel electrophoresis for detection of their resistance pattern whether it was chromosome mediated or plasmid mediated using chromosomal DNA and plasmid DNA respectively.

In Chromosomal DNA electrophoresis, there were multiple bands but in plasmid analysis there were no such bands. So quinolone resistance of Escherichia coli isolated from geriatric age group in this study is chromosomal not plasmid mediated.

DISCUSSION: Escherichia coli is most common cause of acute, uncomplicated urinary tract infection outside hospitals, as well as nosocomial urinary tract infection induced septicaemia. Strains that cause urinary tract infection often originate from the gut of the patient and ascending in nature.[3] Pathogenicity of Escherichia coli is due to different gene-encoding virulence factors (VFs), such as adhesins, toxins etc.[4]

Treatment of urinary tract infection in a geriatric group of population poses several problems due to- i) In elderly patients there are age associated multiple severe underlying disorders ii) early recognition of bacteraemic UTI and prompt, appropriate treatment are critical in reducing the mortality.[5] iii) the extensive and inappropriate use of antimicrobial agents result in the development of antibiotic resistance.[6]

Quinolone is a group of antibiotic which acts by inhibiting enzymatic activities of two members of the topoisomerase class of enzymes, DNA gyrase and topoisomerase IV. DNA gyrase is composed of two A and two B subunits, products of gyrA and gyrB genes, respectively.[7,8] DNA gyrase uniquely catalyzes the introduction of negative super helical twists into closed covalently circular chromosomal and plasmid DNA.

The superhelical state of intracellular DNA is regulated by the actions of DNA gyrase and topoisomerase I, which removes DNA super helical twists but is not inhibited by quinolones. DNA super helicity affects the initiation of DNA replication and transcription of many genes.[9,10]

Quinolones also inhibit the activities of topoisomerase IV, another type 2 topoisomerase that is composed of two subunits encoded by the parC and parE genes. Topoisomerase IV lacks the ability to introduce negative supercoils into DNA, a function that is unique to DNA gyrase.[11]

Quinolone resistance is mediated by i) Mutations in the QRDR of gyrA and parC gene. ii) Mutations in the second topoisomerase gene, parE. iii) Up regulation of efflux pumps, which export quinolones and other antimicrobials out of the bacterial cell.

For example, mutations in the gene encoding a repressor of the acrAB pump genes, acrR, are associated with quinolone resistance.[12] Quinolone resistance can also be acquired horizontally through transferable quinolone resistance (qnr) or other DNA.[13]

In geriatric population urinary tract infection rate is high due to underlying age related problem, associated disorder and immunodepression. Quinolone group of antibiotics are indicated in E.coli induced urinary tract infection. MIC_{90} (μg/ml) value of Escherichia coli against different quinolone antibiotics is shown below.[14]

Bacteria	Nalidixic Acid	Norfloxacin	Pefloxacin	Ciprofloxacin	Ofloxacin	Levofloxacin	Moxifloxacin
E.coli	4	0.12 (0.01–0.5)	0.12–0.25	0.25 (0.015–>128)	0.25 (0.06–0.25)	0.5 (0.025–32)	0.25 (0.016–8)

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Some strains of Escherichia coli exhibit resistance to quinolone antibiotic due to any reasons like i) Mutations in the QRDR of gyrA and parC gene. ii) Mutations in the second topoisomerase gene, parE. iii) upregulation of efflux pumps.[15,16,17,18,19]

In this study we found 73% Escherichia coli isolates were quinolone resistant and their electropherotypes were detected by agarose gel electrophoresis.

One study in Bangalore revealed 79.9% of the uropathogens were resistant to fluoroquinolones. It is plausible that frequent fluoroquinolone prescriptions may be contributing to the observed resistance.[20,21] Aypak et al[22] found that treatment durations were statistically longer than the recommended three-day course when patients were empirically treated with fluoroquinolones due to increased resistance rates, and suggested to discourage the empirical use of fluoroquinolones in UTI.

In conclusion, Fluroquinolone resistant Escherichia coli have emerged as a potential pathogen causing both communities acquired and nosocomial urinary tract infection and monitoring of resistance is necessary to prevent treatment failure and increased morbidity and mortality with UTI.

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Date of Submission: 29/08/2014.
Date of Peer Review: 30/08/2014.
Date of Acceptance: 17/10/2014.
Date of Publishing: 21/10/2014.