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Tet (B) Genedetection Of P. Aeruginosaande. Coli In Burn Patients And Urinary Tract Infection In Thi-Qar Province/ Iraq

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Abstract:

The goal of the present study was to profile the tet (B) gene coded to resistance of tetracycline antibiotic via PCR technique in P. aeruginosa andE. coli isolates which isolated from55 swabs of burn patients and 60 urine samples of patients with Urinary Tract InfectionAl-Hussein Teaching Hospital in Thi-Qar province/ Iraq during the period from March to November, 2020. Only 58.33% isolates were identified asE. coli afterward identified by morphological and biochemical tests. Whereas 43.7% of isolates identified as P. aeruginosa from all burn samples subsequently diagnosis by morphological characteristic on culture media and API20 System. The molecular results showed that the tet (B) genewas found in 93% and 96% of P. aeruginosa andE. coli isolates, respectively.

Keyword: tetB gene, PCR,E. coli, P. aeruginosa

Introduction

Escherichia coli, a member of the family Enterobacteriaceae,constitute part of normal commensal bacterial flora of animals and humans (Tajbakhsh et al.,2016); it was the contributing of amount of infections, like gastroenteritis, cystitis in non-hospitalizedpatients, pneumonia and septicemia of mostly nosocomialorigin (Eisenstein& Zaleznik, 2000). Also ithad acquired resistance to many antibiotics, includingthe tetracycline class of agents; Widespread resistance to the broad-spectrum tetracyclines caused by heavy clinical use and misuse in the human population(Chopra & Roberts, 2001).

Urinary tract infection (UTI) was considered as the most frequent human bacterial infection all over the world(Mao et al., 2012). Also the UTI was predominantly caused by the Colonization of the Gram-negative bacterial species such as E. coli, Klebsiella, Proteus and Pseudomonas (Cheesbrough, 2006).

The Pseudomonas, E. coli isolates and other Gram negative species straightforwardly harbors antibiotic resistant (AMR) genes from oneto another; because of these genes encoding AMR determinants that were carried on mobile genetic elements suchas integrons, plasmids and transposons of some bacterial strains could be transferred to other bacteria strains during contact(Iyer

et al., 2013; Van den Bogaard & Stobberingh, 2000), and which favor the co-transfer of MDR phenotypes between commensals and pathogens, animals, and humans.
The study done by Alemu et al., (2009) showed that several pathogen had established resistance against tetracycline drugs (Beyeneet al., 2015).

P. aeruginosa was a Gram-negative, encapsulated, nonsporulated, and strict aerobic motile rod; It was an opportunistic pathogen, widely exists in various ecosystems and believed to be implemented in several serious human and animal diseases (Birdet al., 2017). P. aeruginosa causes numerous diseases in human and which was one of the major issues particularly pneumonia, associated with physical and physiological stress, leading to significant mortality rates (Bangaret al., 2016).It was one of the most important secondary of the hospital-acquired infection in burnt patients (Bayatet al., 2010), and it was leading to the nosocomial pathogen, causing infections that usually accrue late during hospital stay (Schechneet al., 2009).
P. aeruginosawas naturally more resistant to antibacterialagents than many other Gram-negative species of bacteria, including E. coli (Ramos-Aireset al.,2004). The growing problem of antibiotic resistance among bacterial pathogens and the escalating difficulty in finding new antibiotics were driving the search for a new approach to antibacterial chemotherapy (Gomez and Neyfakh, 2006). The purpose of this study was to detect the tet B gene by PCR technique in P. aeruginosa which recovered from burn samplesand from E. coli isolated from UTI.

Material and Methods

Bacterial samples

A total of urine samples (60) were collected from patients with Urinary Tract Infection, and 55 swabs from burn patients at Al-Hussein Teaching Hospital in Thi-Qar province, during the period from March to November, 2020.The samples were directly streaked on Mac Conkey agar, incubated at 37°C for 24h, and then selected isolated colonies were cultured on selective medium and were further identified by Gram stain and API 20E system.

Extraction of bacterial DNA

The P. aeruginosa and E. coli isolates were inoculated on Brain Heart Infusion broth medium (BHI) (LAB/ United Kingdom) and incubated for 24h at 37°C. The genomic DNA of two bacterialsamples were extracted from a fresh culturein BHI broth by using DNA Bacteria plus kit (Geneaid / Korea) according to the manufacturer's instructions.

PCR detection of tet B geneofP. aeruginosa andE. coli isolates

The specific primer pairs of tet (B)gene as following: forward: 5'- TTCGGCATTCTGAATCTCAC-3' and reverse: 5'- ATGATCTAACCCTCGGTCTC- 3'(Van den Bogaard et al., 2001). The PCR cycling program of thisgene was: the initial denaturation at 94°C for 3minutes, followed by 30cycles including: denaturation at94°C for 60seconds, annealing at 56°C for 90seconds, extension at 72°C for 60seconds and final extension for 10minutes after the last cycle (Abraham & Jefferson, 2010). The visualization of PCR products was showed in 2% agarose gelelectrophoresis and the attendance of a 634bp band as a positive result for tet (B)gene.

Results and discussion

The present results recorded that 35/60 (58.33%) isolates as E. coli after identified by morphological and biochemical tests. While only 24/55 (43.7%) of isolates identified as P. aeruginosa from all burn samples after ward diagnosis and identified by morphological on culture media and API20 System.

The Gram-negative bacteria such as Pseudomonas and E. coli were mostly causes of UTI(Cheesbrough, 2006).The present results was disagreed with results of Moller et al., (2016) showed that (150/71.4%) from 210 urine samples from pateints with UTI identified as E. coli.

The molecular results of tet(B) gene in both current bacterial species were 93% and 96% of P. aeruginosa and E. coli, respectively harbored this gene. The Fig (1) showed the molecular size (634bp) of tet(B) gene in both bacterial species.

The using of PCR technique was one of imperative methods to identify diverse bacterial species such as E. coli; also using to identify Gram positive bacteria such as:Staphy lococcus aureus and other bacterial isolates instead the microbiological methods especially those where there was a potential danger of exposure for humans, such as selective and enrichment media (Buyukcangaz et al, 2013; Degaim et al., 2021)

The genes that encode for resistance against tetracycline including (tetA and tetB) were the key cause of antibiotic resistance in E. coli and other Gram- negative bacteria(Oporto et al., 2019). The emergence of antibiotic resistance among Gram-negative bacteria was a world wide challenge that affects human and animal health, further buttressing the need for intensified surveillance (Bhardwaj et al., 2021). Bacteria might be a reservoir of genes for antibiotic resistance and may play a role inthe distribution of antibiotic resistance to other pathogenic and commensal bacteria (Dehdashtiet al., 2019).In addition, E. coliwas known as a very efficient reservoir for antimicrobial resistance genes and can transfer those genes to other pathogenic bacteria (Huet al., 2016).

The tetracycline-resistant bacteria had been broadly distributed in the environment. The study performed by Rubab, & Oh, (2021) showed that tet gene found in 88% of the E. coliwere positive for this gene; also the present study was in agreement with study of Galarce et al., (2020) reported that the high frequency of tetB was the frequent tetracyclinedeterminant in E. coli isolates.

The results of some studies nearness with results of current study which recorded 95% of E. coli isolates had tetB gene, like: Kamrani et al., (2017) demonstrated that(73.98%) of E. coli carried this gene. While the tetgene was detected in (44%) of E. coli isolates in study performed by (Otto et al., 2002).Alsothe local study done byKadhum & Saleh (2020) showed thattet gene was detected only in (31/50; 77.4%) of E. coli isolates.

The prevalence of E. coli isolates harboring resistance gene (tet) responsible for tetracycline antibiotic was found (65.1%) (Messele et al., 2017).

All three types of tetracycline resistance have evolution aryorigins in the environment, but there were now found widely distributed in commensal and pathogenic bacteria(Abid Al Kareemet al., 2020).

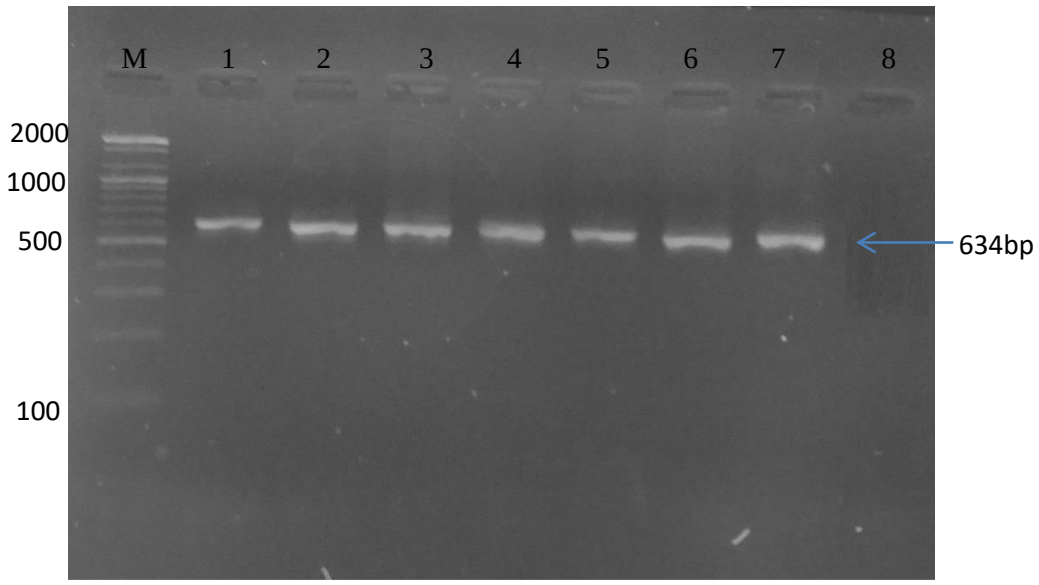

Fig.(1): Agarose gel electrophoresis of tet (B) gene amplification, M: ladder, 1-3 : positive results of this gene inE. coli; 4-7: positive results of this gene in P. aeruginosa; 8: negative result.

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