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Research Article

TEM Gene Detection in Clinical *Pseudomonas aeruginosa* and *Escherichia coli* Samples

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Abstract

Background: Isolation of the *TEM* beta-lactamase gene from clinical *Pseudomonas aeruginosa* and *Escherichia coli* samples provides useful information on the epidemiology of and factors involved in infections caused by these agents as well as their antibiotic resistance patterns. The aim of this study was to evaluate the antibiotic resistance of *P. aeruginosa* and *E. coli* isolated from specimens obtained in Isfahan, Iran via detection of the *TEM* gene.

Materials and methods: In this cross-sectional study, 120 *P. aeruginosa* and 86 *E. coli* samples isolated from urine and sputum were identified using biochemical methods. Their antimicrobial resistance pattern was investigated using the Kirby-Bauer disc diffusion method. Then, phenotypic detection of extended-spectrum beta-lactamases (ESBL) was performed using a combined disc method. Finally, the *TEM* gene in isolated samples was examined using polymerase chain reaction (PCR).

Results: *P. aeruginosa* isolates were found to show the highest resistance to tetracycline (97.5%) and amoxicillin (95%) and the highest sensitivity to aztreonam (97.5%) and amikacin (61.66%). 68 *P. aeruginosa* samples (56.6%) contained a *TEM* gene. *E. coli* isolates were found to show the highest resistance to co-trimoxazole (59.34%) and amoxicillin (55.04%), and the highest sensitivity to imipenem (69.66%) and chloramphenicol (61.92%). 62 *E. coli* samples (72.09%) contained a *TEM* gene.

Conclusions: The alarming spread of ESBL-producing pathogens is a complicating factor in antimicrobial therapies. It is essential to employ diverse strategies for the supervision of the spread of these pathogens.

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1. Introduction

Pseudomonas aeruginosa is a major cause of infections, such as pneumonia, bacteremia, urinary tract infections, as well as cystic fibrosis, in hospitals with weak safety systems (1). *Escherichia coli* is the most common cause of urinary tract infections (2).

E. coli strains are typically divided into four phylogenetic groups: A, B1, B2, and D (3). Uropathogenic *E. coli* (UPEC) were found to be gender-associated and in higher numbers in group B2 (2). Beta-lactam family antibiotics are used to treat infections caused by these two bacterial species. Enzymes called beta-lactamases produced by these bacteria hydrolyze the β-lactam ring of antibiotics and prevent their binding to the target site, causing antibiotic resistance (4). In *E. coli* and *P. aeruginosa* isolates, beta-lactamases were found to be encoded by plasmids (5,6). Extended-spectrum beta-lactamase (ESBL) prevalence levels between 6% and 88% were reported previously in nosocomial infections.

β-lactamases are classified into four molecular classes: A, B, C, and D. Classes A, C and D act via a serine-based mechanism, whereas class B (or MBL: metallo-beta-lactamase) β-lactamases require zinc to function. Plasmid-encoded *TEM-1* β-lactamases are the most studied class A enzymes in gram-negative bacteria. Spreading rapidly throughout the world, they are considered as the most common beta-lactam resistance mechanism in gram-negative bacilli (7,8). Observed *TEM* genes (bla_{TEM}) confer resistance to most antibiotics such as penicillin and first-generation cephalosporins such as cephaloridine. *TEM*-type ESBLs are derived from *TEM-1* and *TEM-2* with amino acid replacements within the active site (9). The global prevalence of bla_{TEM} in clinical isolates varies and has continued to change over time (10). The number of organisms producing *TEM* enzymes continues to increase, and the antibiotic resistance conferred by *TEM* enzymes remains a major crisis in the treatment of infections caused by these bacteria (11). On the contrary, *P. aeruginosa* and *E. coli* are the most important causes of hospital infections, and these pathogenic bacteria show high resistance to a wide range of antimicrobials and antibiotics. Studies on the production of beta-lactamases by these pathogens can provide a relatively adequate insight into antibiotic resistance patterns in a geographical region. Thus, the aim of this study was to determine the antibiotic resistance patterns of *P. aeruginosa* and *E. coli* using the disk diffusion method and to determine the frequency of occurrence of the *TEM* β-lactamase gene in clinical *P. aeruginosa* and *E. coli* samples using polymerase chain reaction (PCR) method.

2. Materials and Methods

2.1. Sample collection and bacterial characterization

Two hundred and twenty clinical samples (urine and sputum) were collected from clinical laboratories in Isfahan province, Iran, between February 2018 and August 2018. A hundred and twenty *P. aeruginosa* isolates and 86 *E. coli* isolates were identified according to basic biochemical tests and Laboratory Standards Institute (CLSI) guidelines. *P. aeruginosa* specimens were collected from people who were susceptible to pneumonia and displayed symptoms, such as difficulty in breathing, shortness of breath, and chest pain. *E. col*i specimens were collected from people with suspected urinary tract infections showing symptoms, such as urinary burning and frequent urination in small amounts.

2.2. Antimicrobial susceptibility test

The phenotypic detection of extended-spectrum beta-lactamases (ESBL) was performed using the double-disk diffusion (DDS) test according to clinical laboratory guidelines. Used antibiotics included chloramphenicol (30 µg), tobramycin (10 µg), co-trimoxazole (25 µg), amikacin (30 µg), ciprofloxacin (5 µg), tetracycline (5 µg), amoxicillin (30 µg), cefotaxime (30 µg), imipenem (10 µg), and aztreonam (30 µg). All discs were obtained from Hi-Media, Mumbai, India. Reference strains of *Escherichia coli* (*ATCC 25922*) and *Pseudomonas aeruginosa* (*ATCC 27853*) were used for the quality control of antimicrobial susceptibility tests.

2.3. DNA extraction and polymerase chain reaction

DNA of the isolates was extracted using a DNA extraction kit (Sinoclon, Iran) according to the manufacturer's protocol. The quality of the extracted DNA was measured using a spectrometer (Thermo Scientific, Waltham, MA, USA). Primer sequences for each bacterium (Table 1) were selected based on previous studies (12, 13). The PCR mixture (Sinoclon) contained 0.6 µl $MgCl_2$ (1.5 mM), 1 µL Taq DNA polymerase (500 U), 5 µl 10x PCR buffer, 0.4 µl dNTP (200 µM), 0.5 µl of each primer (10 pmol/ml), and 2 µl of DNA template (1 µl genomic DNA). The bla_{TEM} gene was amplified under the following conditions: initial denaturation at 95˚C for 5 min followed by 35 cycles including denaturation at 95˚C for 45 s, annealing at 58˚C for 45 s, and extension at 72˚C for 45 s. The cycles were followed by a final extension at 72˚C for 5 min. The PCR products were separated by electrophoresis in 1.5% agarose gel and visualized by staining using green viewer under UV light.

TABLE 1: Primer sequences used for PCR reaction.

Size	Target gene	Sequence	Bacteria species
867	bla_{TEM}	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	*Pseudomonas aeruginosa*
431	bla_{TEM}	AGTGCTGCCATAACCATGAGTG CTGACTCCCCGTCGTGTAGATA	*Escherichia coli*

2.4. Statistical analysis

The results are presented as mean ± standard deviation (SD) of triplicate measurements. The data were analyzed using one-way analysis of variance (ANOVA) using the SPSS software (Chicago, IL, USA).

3. Results

P. aeruginosa isolates were found to show the highest resistance to tetracycline (97.5%) and amoxicillin (95%) and the highest sensitivity to aztreonam (97.5%) and amikacin (61.66%). 68 samples (56.6%) isolated in this study contained a *TEM* gene. *E. coli* isolates were found to show the highest resistance to co-trimoxazole (59.34%) and amoxicillin (55.04%) and the highest sensitivity to imipenem (69.66%) and chloramphenicol (61.92%). 62 samples (72.09%) contained a *TEM* gene (Figure 1 & 2).

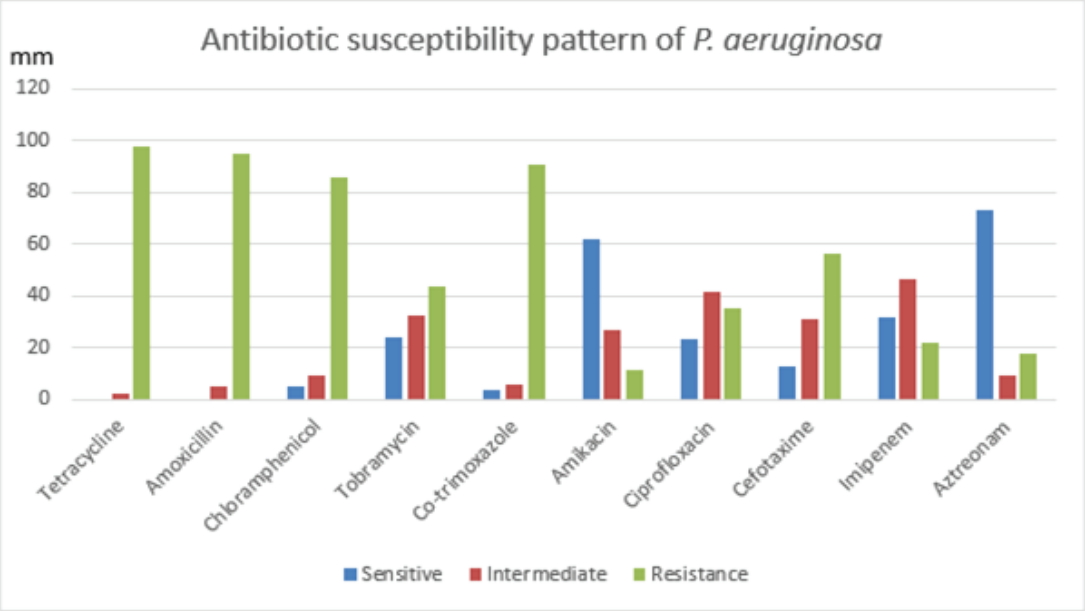

Figure 1: Antibiotic susceptibility pattern *in P. aeruginosa* isolates, inhibition zone diameters (mm).

Our findings showed that 68 (56.6 %) out of a total of 120 *P. aeruginosa* samples and 62 (72.09 %) out of a total of 86 *E. coli* samples carried the *TEM* gene, respectively

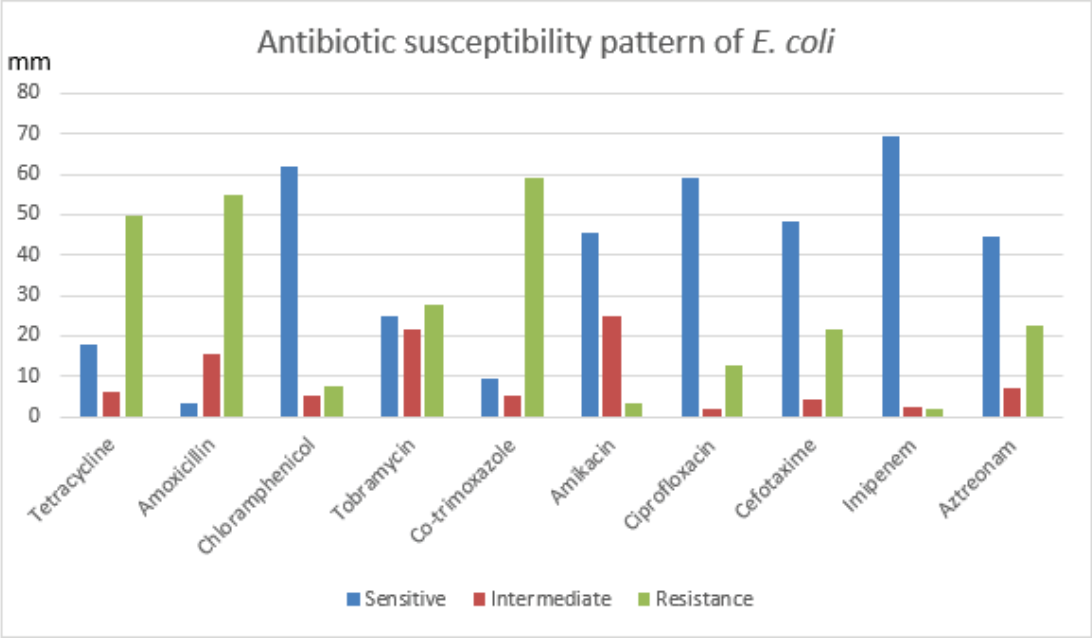

Figure 2: Antibiotic susceptibility pattern *in E. coli* isolates, inhibition zone diameters (mm).

(Figure 3 & 4). The samples with the *TEM* gene showed resistance to all antibiotics to varying extents.

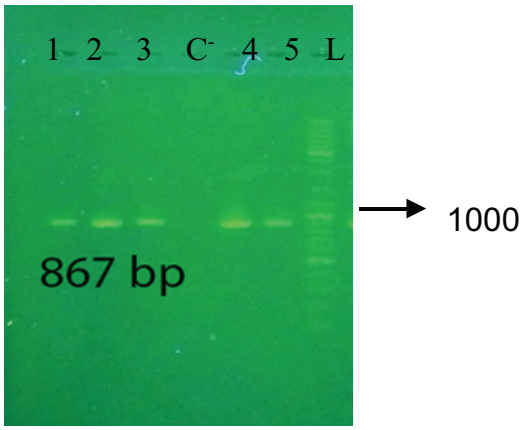

Figure 3: *TEM* amplicon in PCR product of *Pseudomonas aeruginosa* isolates (Column L: Size marker (100 bp), Column C^-: Negative Control, Columns 1, 2, 3, 4, and 5 (867 bp): positive samples).

4. Discussion

P. aeruginosa is one of the pathogens bacteria in hospitals. Most antibiotics used against infections caused by this pathogen are currently are not very effective (14). *E. coli* is still the dominant cause of urinary tract infections worldwide, responsible for 80-90% of urinary tract infections (15). The choice of antibiotic type for the experimental treatment of urinary tract infections is still under debate, as 20-50% of the isolates are currently

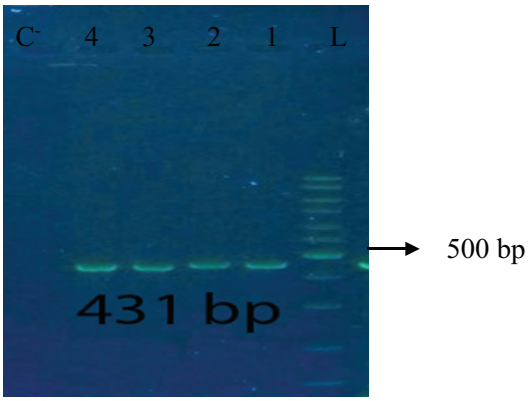

Figure 4: *TEM* amplicon in PCR product of *E. coli* isolates (Column L: Size marker (100 bp), Column C⁻: Negative Control, Columns 1, 2, 3, and 4 (431 bp): positive samples).

resistant to first-line antibiotics, even in developed countries (16). In this study, 220 urine and sputum samples were collected from laboratories in the Isfahan province. Following identity verification tests, the antimicrobial resistance patterns of samples were compared. High levels of resistance to various antibiotics, especially beta-lactams, in *P. aeruginosa* and *E. coli* were found. Most isolated strains were resistant to three or more classes of antimicrobials. Shirehjini et al. previously found high antibiotic resistance levels in *P. aeruginosa* strains and reported that 34.2% of the strains contained a *TEM* gene (17). Peymani et al. reported that the *blaTEM-1* (26.7%) in *P. aeruginosa* strains was the most frequently observed gene, followed by *blaCTX-M-15* (17.3%), *blaSHV-1* (6.7%), and *blaSHV-12* (4%) [18]. Bahrami et al. have investigated the presence of *blaSHV, blaTEM, blaCTX-M* and *blaOXA-48* beta-lactamase genes in 96 clinical isolates of *P. aeruginosa* in Bandar Abbas by the PCR method. The prevalence of *blaCTX-M, blaSHV, blaTEM* and *blaOXA-48* genes were 23.95% (23 isolates), 23.08% (26 isolates), 57.29% (55 isolates), and 12.5% (12 isolate), respectively (19). These findings are clearly close to those reported in this study.

Bajpai et al. determined the prevalence of ESBL (*blaTEM, blaCTX-M*, and *blaSHV*) genes among the members of *Enterobacteriaceae*. 78 *E. coli* and *Klebsiella* isolates were identified out of the 80 members of the Enterobacteriaceae isolated. The prevalence of *TEM* was 55.1% in *E. coli* and 58% in *Klebsiella* (20). Jena et al. have investigated the prevalence of *TEM, SHV*, and *CTX-M* genes of ESBL-producing *E. coli* strains isolated from urinary tract infections. *blaTEM* was the predominant (93.47%) gene followed by *blaCTX-M* (82.6%) and *blaSHV* (4.34%) (21). In another study, Bali et al. showed that among the ESBL-producing isolates of *E. coli*, 72.72% had the *TEM* gene (22). The results of these studies are similar to those reported in the present study. Toupkanlou et al. found that, of the 217 isolates, 87 were cephotaxime resistant gram-negative bacilli. 42 (48.3%) of these were found to be ESBL producers. The prevalence of bla_{SHV}, bla_{TEM},

and *bla*$_{OXA-10}$ genes were 36% among the 50 imipenem-resistant isolates of *P. aeruginosa* (23). These frequencies are clearly close to those reported in this study. Due to the increase of ESBL genes in uropathogens, sustained supervision of the use of antibiotics and that of infection levels are essential. Information regarding the antibiotic resistance patterns of pathogens can assist physicians with the selection of suitable antibiotic regimens. Overall, in this study, the *TEM* gene was identified in more than half of the isolated strains. The alarming spread of ESBL-producing pathogens is a complicating factor in antimicrobial therapies. It is, thus, essential to employ diverse strategies in the supervision of the spread of these pathogens.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

P.B; Contributed to study design. A.E; Contributed to sample collection. B.N, P.B, and E.Sh; Contributed to all experimental work, molecular experiments, and statistical analysis. B.N and E.Sh; Contributed to drafted the manuscript. P.B; Contributed to discussed the findings and approved the manuscript.

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