Current Research in Microbiology and Biotechnology
Vol. 6, No. 3 (2018): 1622-1625
Research Article
Open Access

ISSN: 2320-2246

In vitro susceptibility comparison among different fluoroquinolone generations using *Pseudomonas aeruginosa*

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Received: 01 April 2018 Accepted: 25 April 2018 Online: 01 May 2018

ABSTRACT

A total of 43 *P. aeruginosa* isolates with a frequency of (40.18%) in burn wound patients, were included in this study, Sensitivity test was performed for five quinolone antibiotics (nalidixic acid, Norfloxacin, ofloxacin ciprofloxacin, and levofloxacin), there were significant differences among the activity of those five fluoroquinolones antibiotics, ciprofloxacin and levofloxacin were the most active against *P. aeruginosa,* followed by norfloxacin; fluoroquinolones have lost their spark as none of the currently used quinolones showed resistance levels lower than 50%.

Keywords: *P. aeruginosa*; burn Infection; Resistance.

1. INTRODUCTION

Burn injury, one of the significant public health problems worldwide, is at high risk for nosocomial infections [1]. According to Swedish study, the most common infection was burn wound infection (60%) [2]. *P. aeruginosa* is an opportunistic pathogen causing severe, acute and chronic nosocomial infections in immunocompromised as well as catheterized or burn patients especially in developing countries [3, 4], *P. aeruginosa* infections are problematic due to its intrinsic as well as acquired resistance to many effective groups of antibiotics [5].

Fluoroquinolones are the only accessible antibiotics for effective oral treatment of infections caused by this organism [6]. Among fluoroquinolones, ciprofloxacin and levofloxacin are widely used in the treatment of P. aeruginosa infections [7].

In the United States an increase in use of fluoroquinolones by 40% was established in the 1990s, doubling the rates of resistance to ciprofloxacin among gram-negative bacilli isolated from the intensive care units of hospitals [8].

2. MATERIALS AND METHODS

2.1 Bacterial isolation and Identification

A total of 107 burn swabs were collected between August and December 2016 from different hospitals in Baghdad, Iraq, according to cultural and conventional biochemical test results 56 were supposed to be *P. aeruginosa*, however; only 43 of which were identified as *P. aeruginosa* with 16srDNA.

2.2 DNA Extraction and PCR

The DNA of 56 supposedly *P. aeruginosa* isolates was extracted using a commercial extraction kit (G-spin extraction kit, Intron, Korea), following the manufacturer's instructions, for DNA purification from gram negative bacteria

A PCR reaction with a specific primer (*pass*), for 16srDNA was performed to identify *P. aeruginosa* [9] (Table 1). (25µl) of PCR amplification mixture contained (5 µl) maxime PCR premix (i-taq) (Intron, Korea), (1 µl) forward primer, (1 µl) reverse primer, (15 µl) sterile distilled water, and (3 µl) DNA template.

The protocol for PCR condition was: initial denaturation 94 C̊ for 2 min., denaturation 94 C̊ for 25 sec., annealing 56.4 C̊ for 25 sec., extension 72°C for 40 sec., and final extension 72 C̊ for 5 min.

The bands presence and size were confirmed with gel electrophoresis using agarose gel (1.5%) stained with the red-safe dye, and DNA ladder (1000bp) (Intron, Korea).

Table 1: Forward and reverse primers sequences, length, GC% and PCR product for the identification of *P. aeruginosa*

Primer	Nucleotide sequence (5´ to 3´)	Length	GC%	Predictive PCR product
PASS F	GGGGGATCTTCGGACCTCA	19	63.2%	956 bp
PASS R	TCCTTAGAGTGCCCACCCG	19	63.2%	

2.3 Antibiotic Susceptibility Testing

The susceptibility test using modified Kirby-Bauer disk diffusion method for five different generation fluoroquinolone family antibiotics namely (nalidixic acid, ofloxacin, norfloxacin, ciprofloxacin, and levofloxacin), recommended by the clinical and laboratory standards institute [10], using commercially available disks (Mastdiscs, U.K) was performed.

An overnight culture was adjusted to 0.5 McFarland standard, cultured on Muller Hinton agar (Himedia, India), and incubated at 37°C for 18-24 hr.

3. RESULTS AND DISCUSSION

A total of 107 burn swabs were collected between August and December 2016 from different hospitals in Baghdad, Iraq, 43 of which were identified as *P. aeruginosa* with 16srDNA (Fig.1), results showed that 16srDNA is a more accurate method for the detection of *P. aeruginosa* than biochemical methods [11].

The frequency of *P. aeruginosa* over 5 months from three different hospitals, was (40.18%) this was somewhat similar to the result obtained by [12] in India, higher than a previous local study [13], and a non-local study in egypt [14] but lower than non-local results in Iran [15], India [16], and Palastine [17].

The variability in *P. aeruginosa* isolation percentage may be attributed to geographic, climatic, and hygienic factors among different areas. The high prevalence of *P. aeruginosa* in our community may be related to the elevation of burn patients in our population which may be the result of different increased kitchen mishaps, terrorist attacks, and fires from electrical shortages in our city.

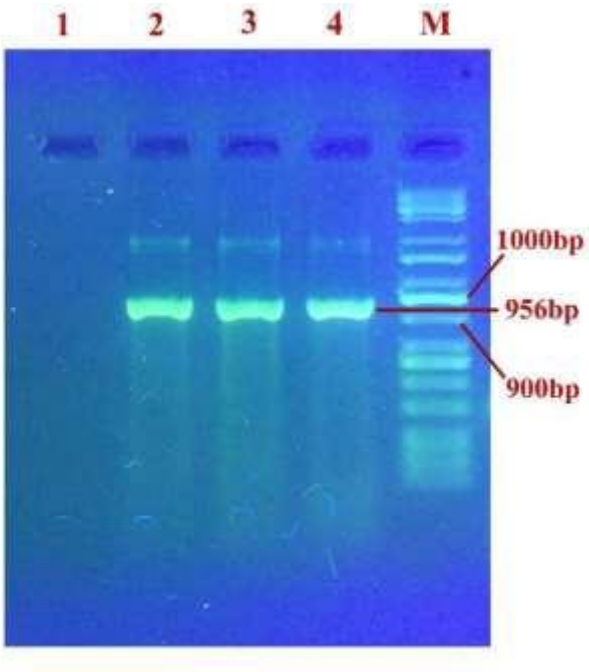

Figure 1: Gel electrophoresis of amplified *pass* (956bp), from *P. aeruginosa* using conventional PCR. Agarose 1.5%, 80 V/cm for 1hrs, stained with safe red dye and visualized on a UV transilluminator. lane 1: Negative control (had all PCR mixture with the substitution of water for DNA template), lane 2-4: Amplicons of *pass* (16srDNA), and M: 100 bp DNA ladder.

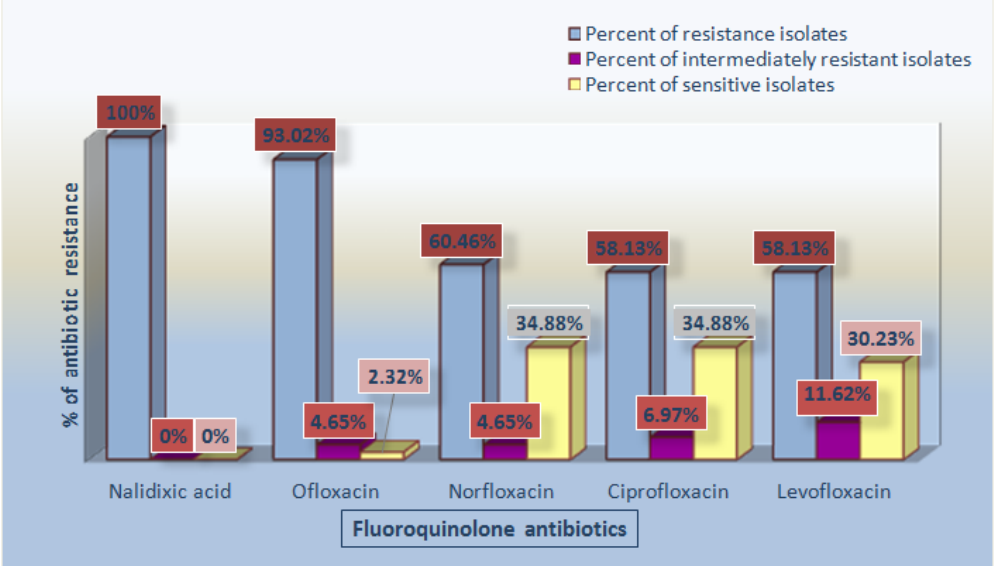

Figure 2: Susceptibility test results for five quinolones antibiotics (nalidixic acid, ofloxacin, norfloxacin, ciprofloxacin, and levofloxacin) against *P. aeruginosa* in percentage

In addition, relaxation in general hygienic measures are associated with increasing infections with these bacteria. The universal nature to survive in the moist environment and resistance to many antibiotics makes *P. aeruginosa* a common pathogen in intensive care units of the hospitals [18].

The resistance patterns of P. aeruginosa were determined (Fig.2), isolates showed varied levels of resistance to the five fluoroquinolones antibiotics: nalidixic acid (100%) resistance, ofloxacin resistance, norfloxacin (60.46%) resistance, and ciprofloxacin and levofloxacin (58.13%) resistance. Ciprofloxacin and levofloxacin were the most active among the tested quinolones [19].

The highest resistance rate was observed in nalidixic acid (100%) of the isolates were resistant, our results of (100%) resistance matched that of local study [20], however, in other local studies the resistance rates were lower than ours, (89%) were resistant [21], (54%) were resistant [22].

The second highest resistance rate was found in ofloxacin (93.02%), no previous local results reported a resistance rate as high as the rate in this study [19, 22], a non-local study showed a higher resistance rate as compared to local studies, but somewhat close to our result [23].

Norfloxacin showed a resistance rate of (60.46%), this result was higher than previous local studies [19,22, 24]. Ciprofloxacin and levofloxacin showed a resistance rate of (58.13%) for each., this is similar to a local study [13], however other previous local studies showed lower resistance rates to ciprofloxacin [24, 25], and lower resistance level to both was shown by [22], non-local studies showed higher resistance rates to both antibiotics [23, 26] Ciprofloxacin and levofloxacin remained the most effective antibiotics within this family.

Multi drug resistant nosocomial infections by *P. aeruginosa* are increasing worldwide, our results showed that fluoroquinolones have lost their spark as none of the currently used quinolones showed resistance levels lower than 50%, and that the percentage of drug resistance has increased in the last couple of years, this can be attributed to the increased misuse of these antibiotics by both hospitals and the community.

4. CONCLUSION

The prevalence of *P. aeruginosa* in burn wound infections has a risen in the last few years. The currently used fluoroquinolones are not as effective as they used to be, as none of them had resistance rates less than 50%. Mutation is the main mechanism of fluoroquinolone resistance among the clinical isolates of P. aeruginosa in Baghdad, Iraq. *gyrA* is the primary target for Fluoroquinolones

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