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Molecular Epidemiology of Antibiotic-Resistant *Escherichia coli* Isolated from Hospitalized Patients with Urinary Tract Infections in Northern Palestine

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Abstract

Eighty isolates of *Escherichia coli* were collected in Northern Palestine throughout the 1996 to 2000 period from hospitalized patients with urinary tract infections (UTIs). Resistance rates were ampicillin, 65%; co-trimoxazole, 55%; cefuroxime, 10%; cefotaxime, 7.5%; ceftazidime, 2.5%; ciprofloxacin, 12.5%; gentamicin, 6.25% and amikacin, 1.25%. No imipenem-resistant isolates were identified. To determine whether this was due to intra-hospital transmission of resistant strains, clonal structure of 10 multiple-resistant isolates was examined by genomic DNA fingerprinting by enterobacterial repetitive intergenic concensus-polymerase chain reaction (ERIC-PCR) and all were clonally distinct. Thus, these strains are likely resistant due to convergent acquisition of resistance determinants by genetically unrelated uropathogenic strains rather than epidemic spread of resistant isolates.

Key words: *E. coli*, antibiotic-resistance, DNA fingerprinting, ERIC-PCR

Introduction

Escherichia coli is recognized as one of the most important bacterial pathogens seriously contributing to the problem of hospital urinary tract infections (UTIs) all over the world. An increase in its resistance to numerous antibiotics has been noted in recent years. The β-lactams, sulphonamides and quinolones are drug families most concerned (Hsueh *et al.*, 2002; Sotto *et al.*, 2001; Okeke *et al.*, 2000; Allen *et al.*, 1999; Perrin *et al.*, 1999; Gupta *et al.*, 1999; Guyot *et al.*, 1999).

The present study aimed to obtain a snapshot of uropathogenic *E.coli* resistance in northern Palestine, a part of the world not previously surveyed for this type of resistance. To find out whether antibiotic resistance was related to intra-hospital transmission of resistant strains, ten multiple-resistant isolates were studied for genetic analysis by ERIC-PCR.

Experimental

Materials and Methods

***Escherichia coli* isolates.** A total of 80 *E. coli* strains isolated from hospitalized patients with urinary tract infections in Rafidya hospital in Nablus, Northern Palestine throughout the 1996 to 2000 period: 28 in 1996, 20 in 1997, 12 in 1998, 10 in 1998 and 10 in 2000 were studied. Reisolates of the same strain from the same patient were not included. The isolates were confirmed as *E. coli* by the API 20E system (bioMérieux, Marcy L'Etoile, France).

Antimicrobial susceptibility. The susceptibility of isolates to antimicrobials was determined by disk diffusion (Bauer *et al.*, 1966) in accordance with National Committee for Clinical Laboratory Standards (1993). The following antibiotics (μg) were used: ampicillin, cefuroxime, cefotaxime, ceftazidime, co-trimoxazole, imipenem, gentamicin, amikacin and ciprofloxacin.

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ERIC-PCR. Ten multiple-resistant isolates, *i.e.*, resistant to ampicillin and two or more of the following antibiotics: co-trimoxazole, ciprofloxacin and aminoglycosides analayzed by ERIC-PCR. PCR was performed with primer ERIC2, 5′AAGTAAGTGAC TGGGGT GAGCG3′ (Dalla Costa *et al.*, 1998) using crude heated isolates in 25-µl reaction mixtures and 5 µl of template DNA. Initial denaturation was carried out at 94°C for 5 min followed by 40 cycles of amplification (denaturation at 94°C for 60 sec, annealing at 25°C for 60 sec, and extension at 72°C for 90 sec) ending with a final extension at 72°C for 5 min. Separated PCR products in agarose gels were visualised as above and patterns that differed by one or more DNA bands were considered to be different ERIC-types.

Electrophoresis. PCR products (25 µl) were mixed with 2 µl of agarose gel loading dye, separated on a 2% agarose gels containing 0.25 µg ethidium bromide per ml and run at 100V for 1 hr. A 100-bp DNA ladder was used as a molecular size marker. Gels were photographed on a 392-nm-wavelength transilluminator and band patterns were compared visually. Patterns that differed by one or more DNA bands were considered as different type.

Results

The rates of resistance of *Escherichia coli* isolates to different antibiotics tested are reported in Table I. Among the antibiotic agents tested in our study, the highest rates of resistance were found for ampicillin (65%), co-trimoxazole (55%) and ciprofloxacin (12.5%). Broad-spectrum cephalosporins remained active, with the rate of resistance to these drugs ranging from 2.5 to 10%. Imipenem was always active. Rates of resistance to aminoglycosides were 6.25% to gentamicin, with amikacin having better activity (rate resistance to amikacin, 1.25%).

Table I
Rates of resistance to different antibiotics tested against 80 *Escherichia coli* strains isolated from urinary tract infections

Antibiotic	No (%) of resistant isolates
Ampicillin	52 (65.0)
Cefuroxime	8 (10.0)
Cefotaxime	6 (7.50)
Ceftazidime	2 (2.50)
Imipenem	0 (000)
Co-trimoxazole	40 (50.0)
Gentamicin	5 (6.25)
Amikacin	1 (1.25)
Ciprofloxacin	10 (12.5)

Table II
ERIC-PCR banding patterns obtained with multiple-resistant *Escherichia coli* isolates and their resistance patterns

Strain no	Year	Resistance pattern[a]	ERIC-PCR Pattern
3	1996	Amp^r, Sxt^r, Ctx^r, Cxm^r, Gen^r	I
4	1996	Amp^r, Ctx^r, Cxm^r, Gen^r	I
12	1996	Amp^r, Sxt^r, Cxm^r, Gen^r	II
38	1997	Amp^r, Sxt^r, Cip^r	III
44	1997	Amp^r, Sxt^r, Cxm^r, Cip^r	VI
55	1998	Amp^r, Sxt^r, Cip^r	V
58	1998	Amp^r, Sxt^r, Ctx^r, Cxm^r, Ctz^r, Cip^r	VI
64	1999	Amp^r, Sxt^r, Ctx^r, Cxm^r, Cip^r	VII
68	1999	Amp^r, Sxt^r Ctx^r, Cxm^r, Ctz^r, Ami^r Cip^r	VIII
75	2000	Amp^r, Sxt^r, Ctx^r, Cxm^r, Gen^r, Cip^r	IX

[a] Amp: ampicillin, Sxt: Co-trimoxazole, Ctx: cefotaxime, Cxm: cefuroxime, Ctz: ceftazidime, Gen: gentamicin, Ami: amikacin, Cip: ciprofloxacin.

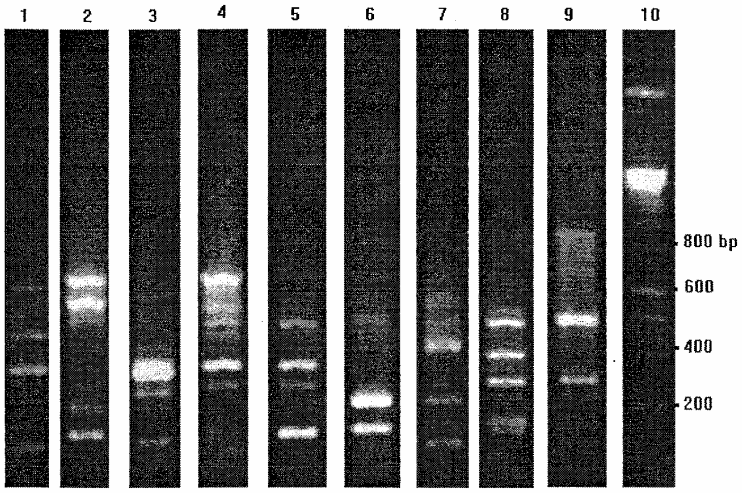

Fig. 1. ERIC-PCR profiles of DNA from *Escherichia coli* isolates.
Lane 1, pattern I; Lane 2, pattern II; Lane 3, pattern III; Lane 4, pattern IV; Lane 5, pattern V; Lane 6, pattern VI; Lane 7, pattern VII; Lane 8, pattern VIII; Lane 9, pattern IX; Lane 10, Molecular sizes marker (100-bp ladder DNA).

Genomic fingerprinting by ERIC-PCR revealed diversity of the 10 multiple-resistant isolates analyzed. The isolates were allocated in 9 genotypes, 8 of which (80%) were represented by single strain. Isolates 3 and 4, although sharing very similar ERIC-PCR patterns were characterized by different antibiogram patterns (Table II and Figure 1).

Discussion

In this study, the *Escherichia coli* isolates showed high degrees of resistance to ampicillin and co-trimoxazole. Comparison of our results with the previous studies shows that there is a rising trend in incidence of resistance to penicillins, sulphonamides and also to the ciprofloxacin (Sotto *et al.*, 2001; Lepelletier *et al.*, 1999; Perrin *et al.*, 1999; Gupta *et al.*, 1999). This probably reflects the fact of the increased usage of these antimicrobial agents, particularly ciprofloxacin, in treating hospitalized patients with urinary tract infections in Rafidya hospital (personal communications). On the other hand, imipenem behaved as the most potent antibiotic. The highest activity of imipenem seems to be related to its stability against most β-lactamases and it is a rapid permeant (Livermore, 1995).

While the resistance to ampicillin and co-trimoxazole is predictable, the high resistance to the ciprofloxacin gives cause for concern. In our study, the rate of resistance to ciprofloxacin was 12.5%. Reported rates of resistance to ciprofloxacin have steadily increased since its introduction and were frequently between 3 and 10% (Sotto *et al.*, 2001; Perrin *et al.*, 1999; Garau *et al.*, 1999). However, rates differed widely from one study to another. For example, Gupta *et al.* (1999, 1994) investigated UTIs in young women who were outpatients and found resistance rates of 0 to 0.2%, whereas others investigators reported rates as high as 20.6% and that 20% of strains from hospitalized patients (Ena *et al.*, 1998; Gruneberg, 1994).

Genomic fingerprinting by ERIC-PCR revealed an extreme diversity of profiles among the multiple-resistant isolates analyzed. Even within isolates sharing similar ERIC-PCR patterns no close relationships in antibiogram patterns were evident. The lack of correlation between antimicrobial resistance patterns and ERIC-PCR patterns of the strains suggests convergent acquisition of resistance determinants by genetically unrelated uropathogenic strains rather than intra-hospital transmission of resistant strains. This probably reflects the fact of the excessive and/or inappropriate usage of these antimicrobial agents, particularly broad-spectrum agents, in treating *E. coli* responsible for urinary tract infections in these health centers.

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