

Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance

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Antibiotic resistance of urinary tract pathogens has increased worldwide. Our aim was to provide information regarding resistance patterns of *Escherichia coli* in urinary tract infections (UTIs) and *E. coli* bacteraemia in Denmark. The overall resistance ranged from: ampicillin 20–47%, mecillinam 0–7%, trimethoprim 10–28%, sulfamethizole 22–47% and nitrofurantoin 0–3%. In strains with sulfamethizole MICs > 2048 mg/L, 97% carried *sull*, *sulll* or both genes, with *sulll* being the most common. Among the *sull* gene-positive strains, 96% were *intl 1* gene positive.

Introduction

The choice of empirical treatment for uncomplicated urinary tract infections (UTIs) is debated, because 20–50% of *Escherichia coli* are now resistant to some of the first-line antibiotics. The present study was designed: (i) to determine the current resistance pattern among *E. coli* UTIs in general practice, hospitalized patients and *E. coli* bacteraemia originating from community-acquired UTIs; (ii) to look at co-resistance; and (iii) to determine the distribution of *sul* genes in Danish isolates. In Denmark the recommended first-line treatment for uncomplicated UTI is sulfamethizole.

Materials and methods

Bacterial strains

Group I. Forty-four general practitioners in Roskilde County each received 20 Uricult Trio dipslides (Orion Diagnostica, Espoo, Finland), from February to April 1997, on which to perform consecutive urinary culturing on all patients with symptoms of UTI. The UTI was defined as uncomplicated if all of the following criteria were fulfilled: (i) female gender; (ii) age 14–60 years; (iii) symptoms: dysuria, frequency, suprapubic pain, absence of loin pain, absence of fever; (iv) not pregnant; (v) no known deformity in the urinary tract; and (vi) less than

three UTIs in the past year. All other UTIs were classified as complicated.¹ All dipslides, including those with $\leq 10^5$ cfu/mL, were sent to Statens Serum Institute, Department of Microbiological R & D.

Group II. The Department of Clinical Biochemistry at the county hospital in Roskilde (RAS) performed (from April to June 1999) all urinalyses at the hospital and forwarded cultivated consecutive strains from hospital-acquired UTIs (defined as UTI starting >3 days after admission).

Group III. A selection of *E. coli* bacteraemia strains from RAS tested at the Department of Clinical Microbiology (DCM) at Statens Serum Institute (1997–1999) was carried out according to the following criteria: probable focus in the urinary tract and infection acquired in the community (bacteraemia <3 days after hospitalization).

Identification

Group I: dipslides were inspected visually, according to the manufacturer's manual, and the cfu/mL was determined on CLED agar, MacConkey agar and *E. coli* agar (β -glucuronidase). Only dipslides with $\geq 10^3$ cfu/mL were processed further, and were cultured on differential media. Identification for groups II and III relied on the identification originally performed at RAS and DCM, respectively.

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Antibiotics

Mecillinam (Leo Pharmaceuticals, Ballerup, Denmark), ampicillin (Sigma Chemical Co.), sulfamethizole (Sigma), trimethoprim (Sigma) and nitrofurantoin (Sigma) were obtained from their respective manufacturers.

MIC determination

MICs were obtained by the agar dilution method according to the NCCLS,² using Mueller–Hinton II agar (Becton Dickinson). The breakpoints used for Enterobacteriaceae were as defined by the NCCLS.³

PCR

All *E. coli* isolates were tested for the presence of sulphonamide resistance genes (*sull*, *sulII*) and integron 1 gene (*int1*). The oligonucleotides were synthesized at TAG Copenhagen A/S, and their sequences were (5'→3'): 16S-F, GCGGACGGT-GAGTAATGT; 16S-B, TCATCCTCTCAGACCAGCTA (200 bp); Sul 1-F, CGGCGTGGGCTACCTGAACG; Sul 1-B, GCCGATCGCGTGAAGTTCCG (433 bp); Sul 2-F, GCGCTCAAGGCAGATGGCATT; Sul 2-B, GCGTTTGA-TACCGGCACCCGT (293 bp); Int-F, GCCACTGCGC-CGTTACCACC; Int-B, GGCCGAGCAGATCCTGCACG (898 bp) (GenBank accession numbers AF071413, M36657, AF071413, AE000452). The PCR mixture contained: 5 µL of template DNA, 5 µL of 10×PCR buffer (Perkin Elmer); 10 µL of dNTP mix (Pharmacia Biotech, Sweden); 4 µL of MgCl₂ (25 mM; Perkin Elmer); 0.25 µL of AmpliTaq DNA polymerase (50 µM; Perkin Elmer); 2.5 µL of each primer 16S-F, 16S-B (40 µM), Sul 1-F, Sul 1-B, Sul 2-F, Sul 2-B, Int-F and Int-B (2 µM); 15.75 µL of distilled water (Gibco-BRL). Amplification (GeneAmp PCR System 2400; Perkin Elmer) was carried out by heating for 5 min at 94°C, followed by 30 cycles of 94°C for 15 s, 69°C for 30 s and 72°C for 60 s,

followed by one cycle at 72°C for 7 min. Gel electrophoresis was carried out on 2% w/v agarose gel (1% NuSieve GTG agarose and 1% SeaKem GTG agarose) (FMC BioProducts, Rockland, ME, USA). D-15 DNA Marker (Novex, San Diego, CA, USA) was used as a marker and *E. coli* NCTC 50001, *E. coli* NCTC 50020 and a susceptible strain from our own collection were used as controls in each PCR run.

Statistical methods

Fisher's exact test was used, with $P < 0.05$ considered as significant.

Results and discussion

Group I: of 188 samples (94% $\geq 10^5$ cfu/mL) 37% were from uncomplicated UTIs (81% *E. coli*) and 63% were isolated from complicated UTIs (68% *E. coli*). Group II: we received 137 identified strains from RAS (55% *E. coli*). Group III: we found 74 *E. coli* isolates that fulfilled the criteria.

Table 1 shows the distribution of resistance in *E. coli*. Resistance to ampicillin, mecillinam and sulfamethizole was significantly higher in the hospital than in the community *E. coli* in total. Trimethoprim and nitrofurantoin resistance were not significantly different in the three strain collections. Trimethoprim and sulfamethizole resistance among the complicated UTIs were significantly higher compared with the uncomplicated UTIs. Compared with a Danish study in general practice published in 1980,⁴ our data show a significant increase in the ampicillin and trimethoprim resistance, a moderate increase in the sulphonamide resistance and a decrease or status quo in the mecillinam and nitrofurantoin resistance in general practice in Denmark. Data from others with respect to uncomplicated UTIs,⁵ and in general practice in total,⁶ support our findings. The high incidence of resistance among hospital UTIs and bacteraemia resembles that from a British survey.⁷

Table 1. Resistance in *E. coli* according to NCCLS breakpoints

| Antibiotic (breakpoint) | Hospital-acquired UTI [% (n/total)] | Bacteraemia [% (n/total)] | Community-acquired UTI | | |
|-----------------------------------|-------------------------------------|---------------------------|------------------------|---------------------------------|-------------------------------|
| | | | total [% (n/total)] | uncomplicated UTI [% (n/total)] | complicated UTI [% (n/total)] |
| Ampicillin (≥ 32 mg/L) | 47 (34/73) ^{a,b} | 42 (31/74) ^b | 29 (42/147) | 20 (12/59) | 34 (30/88) |
| Mecillinam (≥ 32 mg/L) | 7 (5/75) ^a | 0 (0/74) | 1 (2/147) | 0 (0/59) | 2 (2/88) |
| Trimethoprim (≥ 16 mg/L) | 28 (21/74) ^b | 20 (15/74) | 18 (27/147) | 10 (6/59) ^c | 24 (21/88) |
| Sulfamethizole (≥ 512 mg/L) | 47 (34/72) ^{a,b} | 44 (32/73) ^b | 32 (47/147) | 22 (13/59) ^c | 39 (34/88) |
| Nitrofurantoin (≥ 128 mg/L) | 0 (0/74) | 1 (1/74) | 2 (3/144) | 0 (0/58) | 3 (3/86) |

^aSignificantly different from the value in community-acquired UTI (total) ($P < 0.05$).

^bSignificantly different from the value in community-acquired uncomplicated UTI ($P < 0.05$).

^cSignificantly different from the value in community-acquired complicated UTI ($P < 0.05$).

UTIs and antibiotic resistance

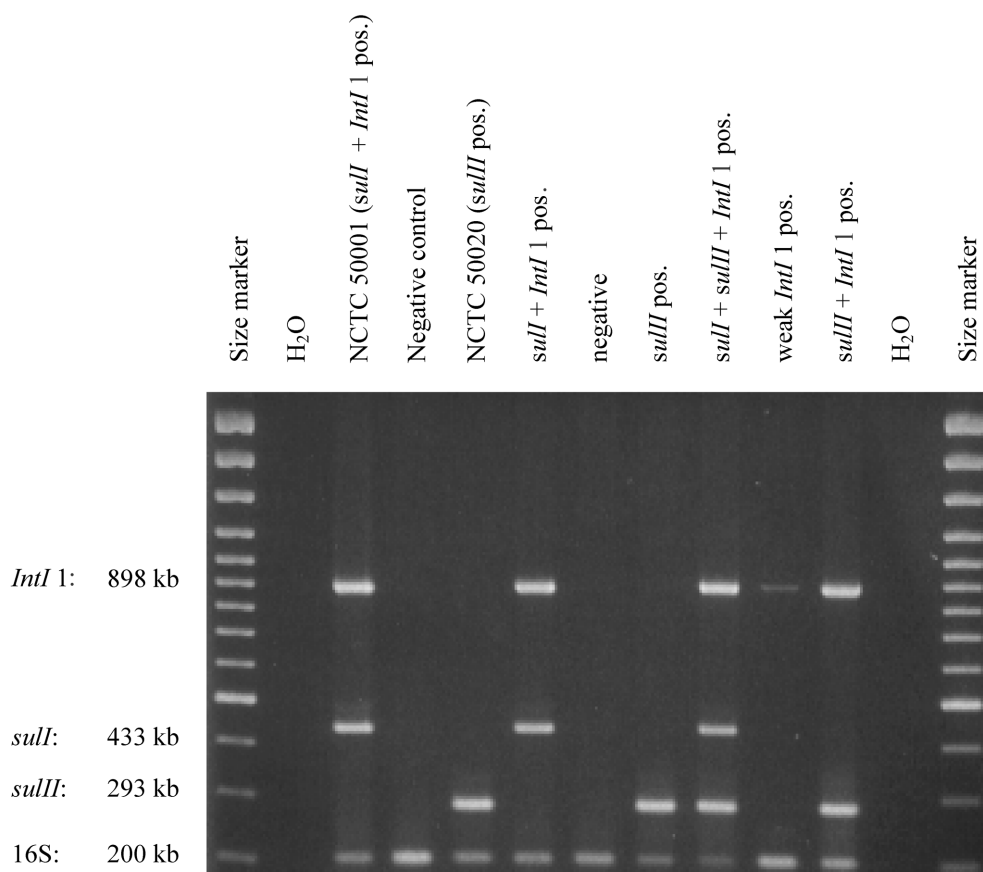


Figure 1. Gel electrophoresis results of multiplex PCR on clinical isolates of *E. coli*.

For strains resistant to either ampicillin, sulphonamide or trimethoprim, resistance to another antibiotic (i.e. co-resistance) was seen in 45–86%. In contrast, in strains susceptible to these antibiotics, co-resistance was seen in only 5–26% ($P < 0.05$).

All 292 *E. coli* were tested for *sul* and *int* genes by the multiplex PCR assay (Figure 1). Strains with MICs ranging from 8 to 512 mg/L ($n = 184$) did not possess any *sul* genes. One *E. coli* with an MIC of 512 mg/L was *sulI* and *sulII* positive. Among the 107 *E. coli* with MICs > 2048 mg/L, the distribution of *sul* genes was as follows: 54 *sulII* positive, 30 *sulI* positive, 20 *sulI* and *sulII* positive; three strains lacked any of these genes. Of the 51 *sulI*-positive isolates, 49 carried the integron-associated integrase gene *IntI* 1. The genetics of clinical sulphonamide-resistant *E. coli* has been published recently,⁸ and the distribution among strains with MICs ≥ 512 mg/L resembles what we have found.

Resistance genes conferring ampicillin, trimethoprim and sulphonamide resistance are linked on R-plasmids that can transfer resistance between bacteria.⁹ In *E. coli*, the sulphonamide resistance conferred by *sulI* (on integron 1) and *sulII* genes has been shown to transfer between *E. coli* in *in vitro* and *in vivo* experiments.^{8,10}

The common opinion in Denmark that sulphonamides and ampicillin are safe to use for empirical treatment of UTIs has

to be re-evaluated in light of the resistance level and the risk of selecting for resistant bacterial populations.

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