Jpn. J. Infect. Dis., 54, 64-68, 2001

Original Article

Prevalence of Resistance to Antimicrobials of *Escherichia coli* Isolates from Clinical Sources at a Private Hospital in Trinidad

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(Received November 28, 2000. Accepted March 1, 2001)

SUMMARY: Antimicrobial susceptibility patterns of strains of *Escherichia coli* isolated between 1994 and 1998 were studied. Of the 1,283 strains examined, 75% were recovered from urine, 8.7% from wounds, 3.2% from blood, 2.6% from pus, and 10.5% from other sources. Isolates from inpatients and outpatients accounted for 46.1% and 53.9%, respectively. Gentamicin and nalidixic acid showed the greatest efficacy against isolates from both inpatients and outpatients, revealing a >90% sensitivity. Drugs with the lowest efficacies were ampicillin and amoxicillin-clavulanic acid, which showed a >45% resistance. Tetracycline showed a significant decline in resistance from 1994 to 1998 among strains from both inpatients and outpatients ($P < 0.001$). This decline may be related to a policy of restrictive antibiotic reporting by the Microbiology Laboratory and seminars for general practitioners, subsequent to an island-wide survey an antibiotic resistance. A similar pattern of declining resistance was also observed for cefuroxime. *E. coli* sensitivity to co-trimoxazole was relatively stable during the study period. Although the overall prevalence of resistance among *E. coli* strains is relatively low, on-going surveillance of bacterial resistance must continue. The microbial antibiogram can provide general practitioners and clinicians with data essential for optimum empiric choices. Further, the introduction of a policy of restrictive reporting may act "synergistically" with the education of doctors on resistance patterns, to effect island-wide reduction of antimicrobial resistance.

INTRODUCTION

The prevalence of antibiotic resistance in *Escherichia coli* strains has been increasing progressively worldwide since the 1970s (1-3). These organisms are among the most frequently isolated in the laboratory as causative agents of infectious diseases. The extent of bacterial resistance to a particular antimicrobial agent varies with the therapeutic practice in that region and among institutions at which the *E. coli* strains were isolated. Variation among *E. coli* resistance patterns from different regions of the world has been previously observed (4-6).

The appearance and rapid spread of resistant organisms have followed the introduction of any new antimicrobial agent (7,8). Although this is the case for many antibiotics, studies have shown relative stability in susceptibility with prudent usage of antibiotics in many areas. In the United States, susceptibility of approximately 98% of *E. coli* strains to ciprofloxacin has been reported, and the aminoglycosides and nitrofurantoin have been shown to be relatively stable for many years (9). A report on antibiotic susceptibility of gram-negative bacteria causing septicemia at 10 Canadian hospitals showed that 98%, 93%, and 91% of isolates (>50% were *E. coli*) remained sensitive to imipenem, aztreonam, and cefazolin, respectively (10). However, the incidence of resistance is higher in developing than in developed countries. In India (11) and Nigeria (12), for example, resistance rates of 64% and 63%, respectively, have been reported for trimethoprim.

Although many studies have indicated that the overall prevalence of bacterial resistance is increasing worldwide, scant documentation exists in Trinidad as to the prevalence of resistance among common isolates, and *E. coli* in particular. The present study was undertaken to assess the extent of antibiotic resistance among local disease-causing strains of *E. coli* in Trinidad, where over-the-counter drugs are readily available and high rates of bacterial diseases exist.

MATERIALS AND METHODS

Source of Specimens: *E. coli* strains from various clinical sources were isolated in the Microbiology Laboratory of the Eric Williams Medical Sciences Complex (EWMSC) between January 1, 1994 and December 31, 1998 (Table 1). The EWMSC is a 560-bed private medical complex located in the northwestern part of Trinidad, the larger of a twin-island republic, Trinidad and Tobago, located about 11 km off the northern coast of Venezuela in South America. The population of the Republic is about 1.25 million. Specimens were obtained from inpatients and outpatients; the former include hospitalized patients, and the latter include those attending the accident and emergency department, outpatient clinics, general practice, and surrounding health centers. Only one isolate with a given resistant phenotype was retained from each patient for antibiotic analysis.

Specimen collection, isolation, and identification: Blood samples came in brain heart infusion (BHI) broth and were incubated at 37°C for an initial period of 48 h. Solution in bottles in which growth was suspected (due to turbidity, gas,

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Table 1. Clinical sources of *Escherichia coli* strains isolated at the EWMSC*, 1994-1998

Nature/Source of specimen	Strains from			
	Inpatients		Outpatients	
	No.	%	No.	%
Urine	398	67.4	561	81.1
Wound swab	64	10.8	48	6.8
Blood	33	5.6	8	1.2
Pus	27	4.6	6	0.9
Sputum	19	3.2	6	0.9
HVS[1]	11	1.9	6	0.9
Stool	5	0.8	13	1.8
Miscellaneous[2]	34	5.7	44	6.4
Total	591	100.0	692	100.0

*EWMSC = Eric Williams Medical Science Complex, [1]HVS = high vaginal swab, [2]Miscellaneous = tissue biopsy, peritoneal dialysate, ear swab.

or hemolysis) was subcultured onto blood, chocolate, and MacConkey agar plates. BHI with negative results were reported after 10 days. Cerebrospinal fluid (CSF), urine (mid-stream and catheter) and sputum specimens were collected in sterile containers. Specimens from wounds, genital tract, pus, and discharge were collected onto sterile cotton Culturette II swabs (Becton Dickinson Microbiology System, Cockeysville, Md., USA). Stool samples came in wide-mouth screw-top plastic sterile containers (Elicey Precision Laboratory Consumables, Shrewbury, Mass., USA).

All specimens (except genital swabs) collected with sterile cotton-tipped applicators (Culturette II) were inoculated onto sheep blood, chocolate, and MacConkey agar plates and incubated aerobically at 35-37°C for 18-24 h. Genital (high vaginal) swabs were inoculated onto chocolate agar and incubated in a CO_2 incubator at 35-37°C for 18-24 h. Bloody fecal specimens were inoculated onto xylose lysine deoxycholate, and both lactose fermenting and lactose non-fermenting colonies were identified. The *E. coli* isolates were subcultured onto sorbitol-MacConkey agar to screen presumptively for the O157: H7 serotype. We were unable to confirm with antiserum that the suspect strains were indeed O157: H7. Urine samples were inoculated onto cysteine lactose electrolyte-deficient and sheep blood agar plates using a platinum wire loop delivering 0.001 ml of urine. A midstream ('clean catch') urine specimen containing 100,000 bacteria per ml or in a catheter specimen containing >3,000 bacteria per ml of a single species was considered as significant bacteriuria (13). Plates were incubated aerobically at 35-37°C for 18-24 h. All specimens were processed according to methods of standard procedure (14). *E. coli* isolates were identified according to colonial morphology, Gram reaction, and biochemical characteristics.

Antimicrobial susceptibility testing: Susceptibility to various antimicrobials was calculated using the agar disc diffusion technique of Bauer et al. (15), on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md., USA), according to the guideline recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (16). Antibiotic discs containing the following concentrations (in brackets) were used: ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), nalidixic acid (30 μg), gentamicin (10 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg) and cefuroxime (30 μg). Antibiotic discs were obtained from several local drug distributors rather than a single supplier. Results were read after 18-24 h and interpreted according to zone size. Intermediate zone sizes were occasional and were excluded from the study. The control organisms were *E. coli* ATCC 25922 strain and *E. coli* ATCC 35218 strain obtained from the Caribbean Epidemiology Center, a branch of the Pan American Health Organization. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Ill., USA).

RESULTS

During the 4-year period, a total of 34,928 specimens were received at the EWMSC, Microbiology Laboratory for culture, of which 16.9% (5,903/34,928) were positive. From these 5,903 positive cultures, 1,283 (21.7%) were common *E. coli* strains. Outpatients accounted for 692 isolates (53.9%) and inpatients for 591 isolates (46.1%) (Table 1). The major sources were urine, wounds, blood, and pus, which together accounted for 1,145 (89.2%) of all isolates.

Table 2a and 2b show the resistance patterns of the strains isolated from inpatients and outpatients, respectively. Gentamicin and nalidixic acid showed the greatest efficacy against isolates in both inpatients and outpatients, showing a >90% sensitivity. The drugs with the lowest efficacy were tetracycline, ampicillin, and amoxicillin-clavulanic acid, in descending order; inpatients showed a <55% sensitivity, and outpatients a <67% sensitivity. Trimethoprim-sulfamethoxazole was relatively stable throughout the period of study in both inpatients and outpatients. In both types of patients, however, cefuroxime showed a gradual but significant decrease in

Table 2a. Percentage of antimicrobial resistance of 1,283 *Escherichia coli* strains isolated from various sources at the EWMSC*, 1994-1998

	Inpatient isolates				
	1994 N = 100	1995 N = 96	1996 N = 118	1997 N = 140	1998 N = 137
% *E. coli* resistant to					
Ampicillin	58.0	47.9	52.5	53.6	60.6
Amoxicillin-clavulanic acid	30.0	21.9	38.1	37.9	27.7
Gentamicin	4.0	5.2	11.9	12.1	8.0
Nalidixic acid	4.0	15.6	3.4	14.3	11.7
Tetracycline	89.0	74.0	44.0	45.2	20.4
Trimethoprim-sulfamethoxazole	20.0	25.0	33.1	56.4	29.9
Cefuroxime	84.0	93.8	12.7	14.3	27.0

*EWMSC = Eric Williams Medical Sciences Complex.

Table 2b. Percentage of antimicrobial resistance of 1,283 *Escherichia coli* strains isolated from various sources at the EWMSC*, 1994-1998

	Outpatient isolates				
	1994 N = 146	1995 N = 106	1996 N = 81	1997 N = 150	198 N = 209
% *E. coli* resistant to					
Ampicillin	53.4	47.2	46.9	64.7	53.1
Amoxicillin-clavulanic acid	29.5	25.5	39.5	27.3	27.8
Gentamicin	15.1	13.2	12.3	13.3	13.4
Nalidixic acid	2.1	7.5	11.1	9.3	6.7
Tetracycline	79.5	79.2	39.5	25.3	41.6
Trimethoprim-sulfamethoxazole	27.4	43.4	27.2	27.3	33.5
Cefuroxime	77.4	86.8	2.5	10.0	35.9

*EWMSC = Eric Williams Medical Sciences Complex.

Table 3a. Proportion (%) of 398 *Escherichia coli* strains isolated from urine specimens resistant to common antibiotics at the EWMSC*, 1994-1998

	Inpatient isolates				
	1994 N = 65	1995 N = 66	1996 N = 68	1997 N = 95	1998 N = 104
% *E. coli* resistant to					
Ampicillin	33.8	33.3	29.4	35.8	40.4
Amoxicillin-clavulanic acid	20.0	13.6	22.1	27.4	19.2
Gentamicin	1.5	4.5	1.5	4.2	2.9
Nalidixic acid	3.1	10.6	13.2	13.7	11.5
Tetracycline	60.0	53.0	26.5	34.7	8.7
Trimethoprim-sulfamethoxazole	29.2	13.6	20.6	37.9	18.3
Cefuroxime	61.5	NT[1]	1.5	7.4	21.2

*EWMSC = Eric Williams Medical Sciences Complex. [1]NT = not tested.

Table 3b. Proportion (%) of 561 *Escherichia coli* strains isolated from urine specimens resistant to common antibiotics at the EWMSC*, 1994-1998

	Outpatient isolates				
	1994 N =114	1995 N = 92	1996 N = 50	1997 N = 130	1998 N = 175
% *E. coli* resistant to					
Ampicillin	43.9	38.0	42.0	54.6	44.0
Amoxicillin-clavulanic acid	24.6	22.8	34.0	33.1	24.0
Gentamicin	1.8	4.3	2.0	3.1	2.9
Nalidixic acid	1.8	6.5	10.0	9.2	6.9
Tetracycline	69.3	63.0	40.0	25.4	28.0
Trimethoprim-sulfamethoxazole	21.1	38.0	26.0	26.9	28.6
Cefuroxime	62.3	42.4	52.0	80.0	85.7

*EWMSC = Eric Williams Medical Sciences Complex.

resistance over the 4-year period ($\chi^2 = 90.75$, $P < 0.001$).

The susceptibility patterns for *E. coli* strains recovered from urine of hospitalized and non-hospitalized patients are shown in Table 3a and 3b, respectively. *E. coli* maintained a relative susceptibility to amoxicillin-clavulanic acid, nalidixic acid, gentamicin, and trimethoprim-sulfamethoxazole throughout the period of study. For tetracycline, resistance rates fell in both types of patients; from 89.1% in 1994 to 20.4% in 1998 in inpatients ($\chi^2 = 95.25$, $P < 0.001$), and from 79.7% in 1994 to 41.6% in 1998 in outpatients ($\chi^2 = 30.41$, $P < 0.001$).

DISCUSSION

The present study found that the frequency of isolation of *E. coli* at the EWMSC (21.7%) among all bacterial isolates was comparable to that reported from Ethiopia (21%) (17) and Kenya (29.2%) (18), but much lower than that reported from Ghana (47.1%) (19).

The prevalence of resistance to ampicillin and amoxicillin-clavulanic acid during the study period was relatively stable. Resistance to ampicillin was generally >50%. This may be due in part to the high prevalence of β-lactamase-producing organisms within the country. In one island-wide study in 1994 by Orrett and Shurland (20), β-lactamase production among 1,572 bacterial isolates from community sources (83% of which were gram-negative organisms) was found to be 73.3%. Resistance to amoxicillin-clavulanic acid was relatively low (32.0%) when compared to that to ampicillin, but considerably higher than reports of 25% and 5.7% from France (21) and Crete (22), respectively. Several mechanisms for resistance to amoxicillin-clavulanic acid among *E. coli* strains have been proposed. These include TEM-1 β-lactamase

hyperproduction (21,23), and increased activity of the SHV-1 β-lactamases, including the CTX M 2-4 group of β-lactamases and the amp C-type β-lactamases such as CMY-2, BIL-1, SAL-1, and LAT-1 and LAT-2 (24). Other resistance mechanisms suggested were the altered permeability of the outer cell membrane (25) and mutations in the genes encoding TEM-1 β-lactamases (26).

In community and hospital situations, *E. coli* strains showed significant improvement in sensitivity to tetracyclines ($P < 0.001$). In 1994, sensitivity among hospital and community strains was 10.9% and 20.3%, respectively. By 1998, sensitivity rose to 79.6% among hospital strains and 58.4% among community strains. Following the survey of Orrett and Shurland (20), a series of seminars were held at the EWMSC along with a large gathering of general practitioners to discuss the efficacy of tetracycline and other antibiotics in treatment of infections. At the EWMSC, a policy of restrictive reporting of tetracycline and other antibiotics by the Microbiology Laboratory was put into effect. This means that if a bacterial isolate is sensitive to, for example, eight antimicrobials, only two would appear on the report sent to the clinician. If the clinician needs more antibiotics, then he/she would discuss same with the microbiogist.Whether these endeavors were directly responsible for the improvement is not clear, though they may have been contributing factors to reduce resistance. Resistance rates of *E. coli* isolates from both inpatients and outpatients to cefuroxime showed a significant decline during the study period. The explanation for this is not altogether clear, though it may be related to erratic importation of the drug and/or infrequent prescribing by doctors.

Although several reports have shown gentamicin resistance to be increasing among enteric gram-negative bacilli, particularly among *E. coli*, resistance to this drug as observed in this study was <10%. Resistance rates of 93% to 100% among gram-negative bacilli have been reported from some hospitals (27-29), and for *E. coli* in particular, >90% resistance to gentamicin was observed (29). In our study, the low prevalence rate may be due in part to our laboratory's policy of restrictive reporting. Further, although gentamicin is not routinely selected for empiric monotherapy, it is usually used in combination with a β-lactam antibiotic, and reports have shown good synergism for this combination (30). Combination therapy with gentamicin has also been shown to prevent the emergence of bacterial resistance to either or both antimicrobial agents (31).

In this study, the relative prevalence of resistance to most antibiotics among *E. coli* isolates was relatively low or stable over the years. However, ongoing surveillance of resistance among all bacteria must continue. Our findings indicated that knowledge of the prevalence of antibiotic resistance provided general practitioners and hospital clinicians with data essential for optimum empiric choices for treating infections (32). Also, introduction of a policy of restrictive reporting from the Microbiology Laboratory may act "synergistically" with education of doctors on resistance patterns, to improve island-wide reduction of antimicrobial resistance. One weakness of this study was that the prevalence of extended–spectrum β-lactamase (ESBL) production among *E. coli* isolates was not included. These ESBLs confer resistance to expanded-spectrum cephalosporins (3rd generation) such as ceftrioxone, ceftazidime, cefotaxime, and the monobactam, aztreonam (33), which were not included in this study because sensitivity data was incomplete for *E. coli* and in some cases absent. The ESBL enzymes are usually encoded by mutated TEM-1 and SHV-1 genes on plasmids, and are easily transmissible from one organism to another (34).

Increased use of the 3rd generation cephalosporins was succeeded by a rising prevalence of resistance to these agents (35). Several institutions have reported increased isolation of ESBL-producing strains of *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter* and *Serratia* spp. from cases of nosocomial infections (36-38). No documented data on the prevalence of ESBL-producing organisms in the Caribbean could be found in the literature. Therefore, the prevalence of ESBL-producing bacteria, in this hospital and the country, is unknown. This is a cause for concern as it has implications for management of serious nosocomial infections.

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