Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia

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Abstract

Background: *Escherichia coli* is the leading cause of urinary tract, ear, wound and other infections in humans. Increasing rates of antimicrobial resistance among *E. coli* is a growing concern worldwide.

Objectives: The aim of this study was to determine the prevalence and antimicrobial susceptibility of *E. coli* from clinical samples.

Method: A retrospective review of culture results of urine, ear discharge, pus swab from wounds, and eye discharge was done. A total of 3,149 samples were analyzed for isolation and identification of bacteria and antimicrobial susceptibility testing.

Results: *E. coli* was isolated from 446 (14.2%) samples. The highest isolation rate was obtained from urine samples 203 (45.5%). High resistance rates to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%) were documented. However, significantly high degree of sensitivity rates to nitrofurantoin (96.4%), norflaxocin (90.6%), gentamicin (79.6%) and ciprofloxacin were recorded ($p<0.001$). Multiple antimicrobial resistances of 74.6% and increased resistance rates to all antimicrobials except ciprofloxacin were also recorded.

Conclusion: *E. coli* isolates showed high rates of resistance to erythromycin, amoxicillin and tetracycline. Nitrofurantoin, norflaxocin, gentamicin and ciprofloxacin are considered appropriate for empirical treatment of *E. coli* in the study area. Regular monitoring of antimicrobial susceptibility is recommended.

Key words: *E. coli*, antimicrobial susceptibility, Ethiopia

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Introduction

Escherichia coli is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation. It is the leading pathogen causing urinary tract infections[1,2,3] and is among the most common pathogens causing blood stream infections[4], wounds, otitis media and other complications in humans[5, 6]. *E. coli* is also the most common cause of food and water-borne human diarrhea worldwide and in developing countries, causing many deaths in children under the age of five years[7].

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Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries[8, 9]. A rise in bacterial resistance to antibiotics complicates treatment of infections. In general, up to 95 % of cases with severe symptoms are treated without bacteriological investigation[10]. Occurrence and susceptibility profiles of *E. coli* show substantial geographic variations as well as significant differences in various populations and environments[11]. In Ethiopia, a number of studies have been done on the prevalence and antimicrobial resistance patterns of *E. coli* from various clinical sources[5, 12, 13]. The aim of this study was to determine antimicrobial susceptibility of *E. coli* from clinical sources at Dessie Regional Health Research Laboratory.

Methods

Study design

A retrospective review was done on results of cultures of urine, ear discharge, pus swab from wounds, and eye discharge that had been performed

from 2003 - 2010 at Dessie Regional Health Research Laboratory. The sex and age of patients. As well as *E. coli* isolates and antimicrobial susceptibility data were collected from the registration records using a standard data collection form.

Culture and identification

The specimens were collected from public and private hospitals and health centers. As the standard operation procedures show, clean-catch midstream morning urine specimens are collected using sterile wide mouth glass containers. Urine samples were plated on Cystine Lactose Electrolyte-Deficient medium (CLED), MacConkey agar and, Blood agar (Oxoid, Basingstoke, UK) using calibrated wire loops and then incubated aerobic atmosphere at 37°C for 24 hours. From positive cultures, uropathogens were identified according to the standard operational procedures as per the standard microbiological methods[14]. A significant bacterium was considered if urine culture yield $\leq 10^5$ colony-forming unit (CFU/ml.

Samples from discharging ears, eye swab, and pus from wound were collected using sterile cotton swabs[14]. Specimens were inoculated onto 5% Sheep's blood agar, chocolate agar, mannitol salt agar and MacConkey agar plates (Oxoid Ltd, Basing stoke Hampshire, UK). The plates were incubated at 37°C aerobically and examined after 24 and 48 hours.

Antimicrobial susceptibility tests

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, Hampshire, England) using Kirby Bauer disk diffusion method[15]. The antimicrobial agents tested were: tetracycline (30 μg), nitrofurantoin (300 μg), erythromycin (15 μg), chloramphenicol (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), cephalotin (30 μg), cotrimoxazole (25 μg), ceftriaxone (30μg), norflaxocin and amoxicillin (10μg) (Oxoid, England). Resistance data were interpreted according to National Committee for Clinical laboratory Standards (NCCLS)[16]. Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control for antimicrobial susceptibility tests[16].

Statistical analysis

The chi-square test was employed to compare the proportion of bacterial isolates with patient sex and age; and comparison of antimicrobial resistances. P-value of < 0.05 was considered to indicate statistically significant differences.

Ethical consideration

Ethical approval was secured from Research Ethics Committee of Bahir Dar University. Permission from Dessie Regional Health Research Laboratory was also obtained.

Results

Between 2003 and 2010, a total of 3149 samples were analyzed for isolation and identification of bacteria and antimicrobial susceptibility testing. *E. coli* was isolated from 446 (14.2%) samples. Of these positive cases, the isolation rate of *E. coli* was the highest in urine samples 203 (45.5%), followed by 146 (32.7%) in ear discharge, 82 (18.7%) in wound swabs and 15 (3.3%) in eye discharge (Table 1).

Table 1: Distribution of *E. coli* from clinical sources at Dessie regional laboratory (2003 - 2010)

Sample	Number of samples tested	Number positive for *E. coli*	% of positive cases
Urine	1404	203/446	45.5
Ear discharge	982	146/446	32.7
Wound swabs	677	82/446	18.7
Eye discharge	86	15/446	3.4
Total	3149	446/3149	14.2

The overall susceptibility patterns of *E. coli* isolates from various clinical sources is displayed in Table 2. Significantly high resistance rates to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%) were documented (p=0. 001). On the other hand, significantly high degree of sensitivity rates to nitrofurantoin (96.4%), norfloxacin (90.6%), gentamicin (79.6%) and ciprofloxacin were detected (p=0. 001).

Table 2: Overall antimicrobial susceptibility patterns of *E. coli* isolated from clinical sources at Dessie regional laboratory (2003 - 2010)

Antimicrobials	Total number of isolates tested	Resistant N (%)	Intermediate N (%)	Sensitive N (%)
Tetracycline	351	254 (72.4)	14 (40)	83 (23.6)
Cotrimoxazole	342	215 (62.9)	8 (2.3)	119 (34.8)
Chloramphenicol	340	120 (35.3)	5 (1.5)	215 (63.2)
Erythromycin	199	178 (89.4)	6 (3.0)	15 (7.5)
Amoxicillin	121	104 (86.0)	0 (0.0)	17 (14)
Cephalotine	148	88 (59.5)	6 (4.1)	54 (36.5)
Ceftriaxone	123	46 (37.4)	1 (0.8)	76 (61.8)
Gentamicin	410	71 (13)	7 (1.7)	332 (81.0)
Ciprofloxacin	186	47 (19.9)	0	139 (74.7)
Norfloxacin	31	2 (6.5)	0	29 (93.5)
Nitrofurantoin	83	3 (3.6)	0	80 (96.4)
p- value		< 0. 001		

As shown in table 3, the overall rate of multiple drug resistance was 74.6% and only 38 (8.5 %) of the isolates were sensitive to eight antimicrobials tested. Site specific multiple antimicrobial resistance rates were 100%, 76.8%, 75.7% and 67.8% for eye discharge, urine, wound swab and ear discharge samples respectively.

Table 3: Multiple antimicrobial resistance patterns of *E. coli* isolates from clinical sources at Dessie regional laboratory (2003 - 2010)

	Antibiogram (No (%)								
Source /Sample	R0	R1	R2	R3	R4	R5	R6	R7	R8
Urine (n=203)	15(7.4)	32(15.8)	53(16.2)	45(22.2)	43(21.1)	11(5.4)	3(1.5)	-	1(0.5)
Ear (n=146)	17(9.6)	30(21.9)	38(25.3)	27(17.1)	18(14.4)	9(5.5)	6(5.5)	1(0.7)	
Wound (n=82)	9(11.0)	11(13.4)	17(20.7)	23(28.0)	14(17.1)	6(7.3)	1(1.2)	1(1.2)	
Eye (n=15)	-	-	1(13.3)	7(40.0)	4(26.7)	2(13.3)	1(6.7)	-	
Total =446	38(8.5)	75(16.8)	109(24.4)	99(22.2)	82(18.4)	27(6.1)	13(2.9)	2(0.4)	1(0.2)

R0= Sensitive to all tested antimicrobials; R1, R2, R3, R4, R5, R6, R8 -Resistant to one, two, three, four, five, six, eight antimicrobials, respectively.

The chi-square test for trend shown in table 4 demonstrates increased resistance rates to seven antimicrobials. In 2003, *E. coli* resistance to tetracycline were 68.4%, to gentamicin 14.3%, chloramphenicol 50.0%, cotrimoxazole 67.2%, cephalotin 56.9%, amoxicillin 76.9% and erythromycin 75.2%. However, in 2010 the resistance rates were tetracycline 76.3 %, gentamicin 13.9%, chloramphenicol 58.1%, cotrimoxazole 68.4%, cephalotin 91.7%, amoxicillin (93.3%) and erythromycin 94.6%, with an increase in rates of 1.2 -34.8%.

Table 4: Chi square test for trend applied to *E. coli* resistance rates to various antimicrobials for eight years at Dessie regional laboratory (2003 - 2010)

	2003		2004		2005		2006		X^2 for trend	p- value
Antimicrobials	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]		
Tetracycline	57	68.4	49	71.4	22	77.3	24	87.5	43.02	<0.001
Gentamicin	63	14.3	61	23.0	55	30.9	33	30.3	47.16	<0.001
Chloramphenicol	52	50.0	50	40.0	34	44.1	15	40.0	60.7	<0.001
Cotrimoxazole	61	67.2	55	58.2	24	58.3	21	76.2	26.94	0.003
Cephalotine	51	56.9	32	53.1	1	100.0	3	100.0	301.6	<0.001
Amoxicillin	13	76.9	32	84.4	20	90.0	17	76.5	260.3	<0.001
Ciprofloxacin	11	36.4	-						77.86	
Erythromycin	4	75.0	4	50.0	18	88.9	17	88.2	68.8	<0.001

Continuation of table 4

	2007		2008		2009		2010		X^2 for trend	p- value
Antimicrobials	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]		
Tetracycline	70	81.4	34	93.82	35	85.7	38	76.3	43.02	<0.001
Gentamicin	75	4.0	56	7.8	37	21.6	36	13.9	47.16	<0.001
Chloramphenicol	71	16.9	50	33	31	21.6	37	58.1	60.7	<0.001
Cotrimoxazole	63	71.4	49	49.34	28	67.9	38	68.4	26.94	0.003
Cephalotine	23	56.5	7	31.4	8	12.5	24	91.7	301.6	<0.001
Amoxicillin	7	100.0	2	55	2	50.0	30	93.3	260.3	<0.001
Ciprofloxacin	358.6	43	32.6	36	11.1	11	17.1	77.86		
Erythromycin	41	87.8	45	92.9	32	100.0	37	94.6	68.8	<0.001

a = number of isolates tested against each antimicrobial agent
b = Percent of isolates resistant to the antimicrobial agent

Discussion

Antimicrobial resistance in *E. coli* has increased worldwide and its susceptibility patterns show substantial geographic variation as well as differences in population and environment[17]. The isolation rate of *E. coli* in the present study was 14.2% and it was commonly isolated from urine samples (45.5%). These findings are in conformity with reports by other researchers[13, 18, 19].

In this study, the overall resistance of *E. coli* to antimicrobials was high. The result is consistent with the findings of previous studies[20]. The resistance rates recorded in this study are higher than the results of Khan et al. [6] and lower than the results of Iqbal and Patel [21] and Okonko *et al.* [22]. High level of resistance in *E. coli* was reported to tetracycline from a study conducted in Ethiopia[23] and to erythromycin from a study done in Slovenia[24].

In all clinical samples, *E. coli* showed high resistance rates of > 80% to erythromycin and amoxicillin and > 60% to tetracycline. The results of this study are in line with the findings of other studies conducted in different parts of the world[25, 26]. However, the antimicrobial resistance rates obtained in this study were higher compared to susceptibility patterns reported from previous studies[27,28,29].

E. coli isolates were sensitive to gentamicin, nitrofurantoin, ciprofloxacin and chloramphenicol. Similar studies conducted in Ethiopia[30] and Nigeria[31] have reported comparable susceptibility rates. High sensitivity to ciprofloxacin and gentamicin and norfloxacin have been recorded from previous studies conducted in Nigeria and India[31,32]. In this study, norfloxacin, ciprofloxacin, gentamicin and chloramphenicol were found to be the most effective antimicrobials against *E. coli* isolates.

Furthermore n this study, a high rate of multiple antimicrobial resistance was recorded, which is consistent with the reports of studies done elsewhere[21,33]. The chi-square test for trend demonstrated increased resistance rates to all antimicrobials except ciprofloxacin. Increases in rates of resistance to different antimicrobials have been reported from previous studies conducted in different parts of the world[20, 33, 34].

Conclusion

The results of this study show high rates of antimicrobial resistance to erythromycin, amoxicillin and tetracycline. Nitrofurantoin, norfloxacin gentamicin and ciprofloxacin are considered appropriate for empirical treatment of *E. coli* in the study area. Periodic monitoring of antimicrobial susceptibility both in the community and hospital settings is recommended.

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