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RESISTANCE PATTERNS OF MULTI-DRUG RESISTANT ESCHERICHIA COLI CAUSING URINARY TRACT INFECTION

Samiya Sabea Khamees [*1], Khadija Saeed Ali Ghafir [2]
[*1, 2] Biology Department, Faculty of Science & Tobruk University /Libya

Abstract

480 mid-stream urine samples were collected from outpatients and inpatients attending Tobruk Medical Center. 300 samples gave positive culture, 162 of these were Escherichia coli.
The strains sensitivity to 14 antibiotics was tested. High incidence of resistant strains, high degree of association between resistance and complex resistance patterns of the same strains is seen. The strains were resistant to Ampicillin, Penicillin, Erythromycin, Cephalexin and Sulfamethoxazole with 91.9%, 90.7%, 82.7%, 76.5% and 57.4% respectively. The strains were sensitive to Neomycin, Oxacillin and Nitrofurtoin with 100%, 99% and 83% respectively. A symmetrical result for all strains resistance to indicated pairs of drugs were obtained. Strong association between most antibiotic occurred.

Keywords: Escherichia Coli; Antibiotics; Resistance Patterns; Urinary Tract Infection.

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1. Introduction

The large intestine constitutes the main source of *E.coli*, which is the principle cause of variety infections including urinary tract infection. Urinary Tract Infection (UTI) is one of the most distributed bacterial infection in many developing countries where good sanitation is not maintained Tanzina *et al*., (2016). It has been reported that up to 15 million person are infected each year worldwide Stam and Norrby, (2001). Antimicrobial resistance has been considered as rising risk both in developed and developing countries (Pfaller; 2000). This risk could be sever in developing countries, where there is no strict information program concerning the use of antibiotics (Jigna and Pratibha, 2012). There are world wide data show that there is increasing resistance among UTI causative agents to the most common prescribed antibiotics, and resistance has emerged even to newest and most efficient antibiotics (Taneja *et al*., 2008).

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The most important antibiotic resistance risks are reported in *Enterobacteraceae* for all major anti-gram negative agents such as beta-lactam, fluoroquinolones and aminoglycosides (Osterblat *et al*., 2000).

Multidrug resistance in *E.coli* is of particular concern because it is the most common gram-negative pathogen causing many infections including UTI. In addition, resistant *E.coli* strains have the ability to exchange antibiotics resistance factor (R-Factor) to other strains of *E.coli* but also to other gram-negative pathogens (Jiana and Pratibha, 2012).

Because now day's antibiotics resistance has become an important phenomenon due to widespread use of antibiotics by patient without testing antibiogram (Tanzia*et al*., 2016).

It is obligatory to have knowledge about the organisms causing UTI and there susceptibility to antibiotics. Therefore, the purpose of this study to isolate, and identify *E.coli* strains from urine samples and determine their susceptibility to common available antibiotics which may help the physician to choose the right treatment for the prevention of UTI.

2. Materials and Methods

Mid-stream urine samples were collected from 480 inpatients and outpatients having symptom suggestive of urinary tract infection attending Tobruk Medical Centre – Tobruk – Libya during the one-year period. The specimens were taken on midday and cultured immediately on MacConkey agar and blood agar by standard culture techniques.

Identification of the isolated organism was done on the basis of cultural characteristic and gram staining was performed to confirm the gram-negative bacilli. Apart from colony morphology, the routine biochemical tests were performed using API 20 system for *Enterobacteraciae*.

The resistance patterns of *E.coli* isolated strains to 14 antibiotics was determined by using disc-diffusing methods and interpreted according to the Nccls (National committee for clinical Laboratory Subcommittee 1990). The following antimicrobial agents were obtained from (Oxoid, UK), Ampicillin, Amoxicillin/calvunic acid, Chloramphenicol, Cefrixon, Cephalexin, Gentamycin, Tetracycline, Nitrofurtoin, Sulfamethoxazole, Erythromycin, Penicillin, Cloxacillin, Oxacillin and Neomycin.

All these tests were performed on Muller-Hinton agar (Oxoid U.k). A 0.05 McFarland Suspension was applied to the plates, which were dried in an incubator at 35c◦for 15 minutes, antimicrobial disc was placed on the agar with sterile forceps. The agar plates were incubated at 35c◦ for 15 hrs. The antibiotics effect for each antibiotics was measured as the diameter of the zone of inhibition.

3. Results and Discussion

Of the 480 urine samples tested from November 2015 to December 2016, 300 urine samples showed positive growth 162 (54%) were E.coli.(Table-1).

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Table 1: Species of Gram-negative Bacteria Isolated from Urine Samples

Species of Bacteria	Number	%
Pseudomonas aeruginosa	26	8.666667
Klebsiella pneumonia	70	23.33333
Escherichia coli	162	54
Proteus mirabilis	42	14
Total	300	100

E.coli strains were collectively tested for sensitivity and resistance against 14 antibiotics (Table - 2). The antibiotics susceptibility of the isolates showed that *E.coli* strains were sensitive to Neomycin, Oxacillin and Nitrofurantoin with 100%, 99%, and 83% respectively.

Table 2: Sensitive and Resistance of *Escherichia coli* Isolated from Urine Samples

Antibiotic	Resistance(R)	%	Sensitivity(S)	%
Ampicillin	149	91.97531	13	8.024691
Amoxicillin/ Clavulanic acid	54	33.33333	108	66.66667
Chloramphenicol	64	39.50617	98	60.49383
Cefriaxon	91	56.17284	71	43.82716
Cephalexin	124	76.54321	38	23.45679
Gentamycin	86	53.08642	76	46.91358
Tetracycline	82	50.61728	80	49.38272
Nitrofurantoin	27	16.66667	135	83.33333
Sulfamethoxazole	93	57.40741	69	42.59259
Erythromycin	134	82.71605	28	17.28395
penicillin	147	90.74074	15	9.259259
Cloxacillin	90	55.55556	72	44.44444
Oxacillin	1	0.617284	161	99.38272
Neomycin	0	0	162	100

Ampicillin (AMP), Amoxicillin/Clavulanic acid (AMC), Chloramphenicol (C), Cefriaxon (CR), Cephalexin (CL), Gentamycin (CN), Tetracycline (TE), Nitrofurantoin (F), Sulphamethaxazole(SXT), Erythromycin (E), penicillin (P), Cloxacillin (OB), Neomycin (N)

The sensitivity result of *E.coli* to the aminoglycoside antibiotic Neomycin with100 percentage and Gentamicin 46.9% was not agreed with the result of Tanzina *et al*., 2016 as they reported that *E.coli* strains were sensitive to the aminoglycosides antibiotics gentamycin and Amikacin with 100%. The result of this study showed that *E.coli* isolates were sensitive to Nitrofurantoin; this was similar to the result of Niranjan and Malini, 2014 and Nalini *et al*., 2014. However, (Tanzina *et al*., 2016) reported that *E.coli* isolates in their study were sensitive to Nitrofurantoin with 93%. Sensitivity of *E.coli* strains to Nitrofurantoin may indicate the narrow spectrum of activity and limited contact of this antibiotic with bacteria outside the urinary tract (Karlowsky *et al*., 2002) or probably due to the non-usage of these antibiotics for long period. Nitrofurantoin has been less commonly used in treatment of UTI infection in recent years (Nalini *et al*., 2014). Other antibiotic, which found effective against *E.coli* in this study, was Oxacillin, as 99.3% of *E.coli* strains were sensitive to this antibiotic. Augmentin (Amoxicillin/calvunic acid) has been known to be efficient

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drug against UTI isolates and its resistance was found to be low as we found only 33.3% of *E.coli* were resistant. Our result was not in agreement with the results of others (Shafaq *et al*.,2011; Taneja *et al*., 2007; Mehrgan and Rahbar 2008) as they reported that their isolates were resistant to Amoxicillin/ calvunic acid with 85%, 95%, and 70% respectively.

The result of this study revealed that *E.coli* isolates were resistant to Ampicillin and Penicillin with 91.9% and 90.7% respectively. In addition, they were resistant to Erythromycin with 82.7%. This result was consistent with other studies carried by Mejbah *et al*., 2011 and Alessandra *et al*., 2011.

This high resistance rate may be due to the widespread and prolonged use of these antimicrobial drugs in the world including Libya. High resistance rate also recorded in this study for the first and third generation Cephalosporin antibiotics Cephalexin and ceftriaxone with 76.5% and 56% respectively. This result was in agreement with the result of Nalini *et al*., 2014 concerning Ceftriaxone and different from the result of Tanzina *et al*., 2016, they reported that *E.coli* strains were sensitive to Ceftriaxone with 63%. However, Mohamed, 2010 reported that *E.coli* isolates were sensitive to cephalexin and ceftriaxone with 20.9% and 11.7% respectively.

Tetracycline resistance is increasing in clinical isolates of *E.coli*. In our study, 50.6% of *E.coli* isolates were resistance to this antibiotic and was less than the results of Shfaq *et al*., 2011 and Noor *et al*., 2004 as their isolates were resistant to Tetracycline with 95% and 83.9% respectively. Although *E.coli* isolates in this study showed resistance to beta-lactam antibiotics, Ampicillin and Penicillin being the most ineffective drug in addition they showed resistance to Cloxacillin with 55.5% whereas one of the most effective antibiotics in this study Oxacillin (99.9%) also belong to the same class. This result is consistent with the result of Rabia *et al*., 2012 for Imipenem (100%), Meropenem 99.3 %, Piperacillin/Tazobactum 96.8%.

Table 3: Matrix Indicating Number of *Escherichia coli* to two Antibiotic at the Same Time.

N	0	0	0	0	0	0	0	0	0	0	0	0	0	-
OX	1	1	0	1	1	0	0	0	1	0	1	1	-	0
OB	77	44	57	66	70	59	39	17	49	85	78	-	1	0
P	140	49	57	86	112	74	75	21	84	121	-	78	1	0
E	123	50	61	82	101	79	63	22	73	-	121	85	0	0
SXT	53	23	28	50	77	59	45	18	-	73	84	49	1	0
F	25	15	16	15	25	20	16	-	18	22	21	17	0	0
TE	75	29	31	47	65	41	-	16	45	63	75	39	0	0
CN	74	33	42	62	72	-	41	20	59	79	74	59	0	0
CL	117	42	55	91	-	72	65	25	77	101	112	70	1	0
CR	88	43	54	-	91	62	47	15	50	82	86	66	1	0
C	57	44	-	54	55	42	31	16	28	61	57	57	0	0
AMC	49	-	44	43	42	33	29	15	23	50	49	44	1	0
AMP	-	49	57	88	117	74	75	25	53	123	140	77	1	0
	AMP	AMC	C	CR	CL	CN	TE	F	SXT	E	P	OB	OX	N

Ampicillin (AMP), Amoxicillin/Clavulanic acid (AMC), Chloramphenicol(C),Cefriaxon (CR), Cephalexin(CL), Gentamycin(CN), Tetracycline(TE), Nitrofurantoin(F), Sulphamethaxazole(SXT), Erythromycin (E) , penicillin (P) , Cloxacillin (OB) , Neomycin (N).

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In our study 99.3%, isolates of *E.coli* were multidrug resistant (Table4, fig1), only one isolate was resistant to two antibiotics. This result is consistent with the result of Shafaq *et al*., (2011),who reported that all *E.coli* Strains in their study were multidrug resistant. This is quite high when compared to other studies. Prevalence of MDR *E.coli* was about 76.5% in a study done by Niranjan and Malini 2014;and 52.9% in study done by Hasan *et al*., 2007only 8.4% and 7.1% MDR *E.coli* reported by Mathai *et al*., 2008 and Sahm *et al*., 200.In this study 95% of *E.coli* isolates were resistant to more than 3 antibiotics belonging to3 or more classes of antibiotics. However in our previous study Khamees, (2001), reported that 94.6% of *E.coli* strains were resistant to one antibiotic and more.

Table 4: The Frequency of Resistance Pattern of Escherichia coli Strains.

Resistance Pattern	**Frequency Number**
AMP-AMC-C-CR-CL-CN-TE-F-SXT-E-P-OB	3
AMP-AMC-CR-CL-CN- TE-F-SXT- E-P-OB	4
AMP-AMC -C-CR-CL-TE-F-SXT-E-P	11
AMC-C-CR-CL-CN-TE-SXT-E-P-OB	10
AMP-AMC-CR-CL-CN-SXT-E-P-OB	5
AMP-AMC-C-CR-CL-CN-E-P-OB	6
AMP-C-CR-CL-CN-TE-SXT-E-P	6
AMC-CR-C-CL-CN-SXT-F-P	5
AMP-CR-CL-CN-SXT-E-P-OB	10
AMP-AMC-CR-CN-SXT-P-OB	8
AMP-AMC-C-CR-E-P-OB	6
AMC-CR-CL-CN-TE-E-P	5
AMP-CR-CL-CN-TE-SXT-P	6
AMP-AMC-CL-TE-E-P-OB	7
AMP-CR-CL-CN-TE-P	9
AMP-CL-CN-SXT-E-P	10
AMP-CL-F-SXT-E-P	10

The increasing prevalence multidrug resistant pathogens is of considerable concern and worrisome as being public health and therapeutic challenge to clinical all over the world. Most encountered gram negative pathogens e.g. *Klebsiella pneumonia* and *E.coli* have the ability to acquire cross resistance to several antimicrobial agents (Khamees and Ghafir, 2017).In our study 60% of *E.coli* isolates showed multidrug resistance to 5-8 antibiotics (belong in to 3 or < 3 different classes of drugs). F.g-1

Table 5: The resistance patterns of *Escherichia coli* strains to more than one drug

Resistance patterns (number of drugs)	**Frequency**	**Total No (%)**
2	1	20(12)
3	7	
4	12	
5	21	97(60)
6	29	
7	24	
8	23	

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9	17	45(28)
10	21	
11	4	
12	3	

These results are comparable to those studies found in Sudan by Ahmed *et al*., 2000; and Rabia *et al.,* 2012 in Pakistan. Also 28% of the isolates from our study showed resistance to more than 8 drugs similarly (AL-Mardeni *et al.,* 2009) in Jordan reported that 59.9% of *E.coli* isolates were resistant to three or more antibiotics. In addition, a study done in Saudi Arabia by Al-Tawafiq., (2006) reported 29% *E.coli* isolates were resistant to more than two antibiotics. However in study done by (Saeed *et al.*,2009) in Karachi, Pakistan 92% of gram negative clinical isolates including *E.coli* resistant to one or more antibiotics. All these results from different countries report more or less the same results.

4. Conclusion

A total of 162 *E.coli* strains were isolated from UTI positive urine samples . 99.3% of these isolated were multidrug resistant to 3 antibiotics and more from different classes only one strain was resistant to two antibiotics different resistant pattern occurred 3-10 times obtained.

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*Corresponding author.
E-mail address: alwan _sami2005@ yahoo.com