Current Science International Volume : 08 | Issue : 03| July- Sept.| 2019
ISSN: 2077-4435 Pages**:** 509-517

Antibiotic sensitivity of *Escherichia coli* isolated from different clinical specimens of patient human in Al- jala hospital- Benghazi Libya

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Received: 10 June 2019/ Accepted 22 July 2019 / Publication date: 30 July 2019

ABSTRACT

Antimicrobial resistance is majorly a problem of public health worry. The aim of this study to isolate and identify *Escherichia coli* from 422 clinical specimens of csf, fluid, pus, sputum swab, tip and urine collected from the clinical locations at Al- jala hospital- Benghazi. The clinical specimens were analyzed using standard microbiological. Common bacteria detected from different Clinical specimens on Selective media. TSI (Triple Sugar Iron), MIU (Motility, Indol, and Urea) *E. coli* followed by *Staphylococcus* and *Pseudomonas. E. coli* isolates were dominant detected in different Clinical specimens on selective media. It was determined antibiotic susceptibility pattern. *The* isolated *E. coli* was microbiological and biochemical identified by Vitek 2 system. Distribution of *E. coli* isolates were differed among Female was 245 (58%) and male 177 (42%). The clinical specimens of urine was the highest *E. coli* 176 (42%) followed by swab 139 (33%) and tip 84 (20%) while the lowest was recorded in csf 1(0%). The studies showed that all the *E. coli* isolated were resistant to. Amikacin, Impienem and Colistin and resistant was observed toward Aug, Septrin and Ciprofloxacin. Effective hygiene must be encouraged and unselective usage of antibiotics must be avoided.

***Keywords*:** Antibiotics, susceptibility pattern, Clinical, *Escherichia coli.*

Introduction

Forty patients showing different types of infections were included, 92 samples were collected which included blood, urine, swab, sputum, pus, and endotracheal tube (ETT) samples were found positive, i.e. showed the growth of microorganisms. (*Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., *Staphylococcus aureus*, *Enterococcus*, *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., and *Candida* spp. (Shamim *et al.,* 2018). *Escherichia coli* is a gram-negative facultative anaerobic bacillus that belongs to the Escherichia genus, which is made up of species present in the human and other animal intestine, it does not survive outside the animal body When eliminated in the environment together with feces it contaminates water, soil and food (Buiuc *et al.,* 1999 an Debeleac Lucia, 2003). *E. coli* tends to affect the intestine and the urinary tract but almost any extra-intestinal site may be involved (Torok *et al.,* 2010 a&b) which consists of colonization of a mucosal site, evasion of host defenses, multiplication and host damage.(Torok *et al.,* 2010b and Cooper, 2003) . Six types of pathogenic *E. coli* strains are of associated with diarrhea: shiga toxin-producing (STEC) or enterohemorrhagic (EHEC); enterotoxigenic (ETEC); enteropathogenic (EPEC); enteroaggregative (EAEC), enteroinvasive (EIEC) and diffusely adherent *E. coli* (DAEC) (Torok *et al.,* 2010 a&b) two billion cases of acute diarrhea are calculated to occur yearly. This disease is the second cause of child mortality the main causes of illness and death in children in the developing countries, with children under 5 years of age having a mean 3 diarrheic periods per year.10 In general, monotherapy with trimethoprim sulfamethoxazole, aminoglycosides, cephalosporin or flouroquinolones is recommended as the treatment of choice for most known infection with *E. coli*, though many broad spectrum agents (such as beta-lactam/beta lactamase inhibitor combination and the carbapenems remain highly active (Kibret and Abera , 2011 and Croxen *et al.,* 2013).

In this study we aimed at screening and assessing the antibiotic susceptibility pattern of *E. coli* isolated from clinical specimens in Al-jala hospital-Benghazi city.

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Materials and Methods

Clinical specimens:

The different clinical specimens were collected from, csf and fluid. pus, sputum , swab , tip and urine from Aljala hospital during (January 2017-Decamber 2018). Each of specimens were inoculated individually on Blood Agar, chocolate agar Macconky agar, CLED Agar, S S agar , TSI (Triple Sugar Iron), MIU (Motility, Indol, and Urea) and Citrate agar and incubated for 16–24 h at 37ºC (ARHAI, 2014)

Detection and Isolation of *E. coli*:

Using multiple tube technique was used for further *E. coli* confirmation. Lauryl tryptose broth with MUG medium was used in test tubes containing inverted fermentation vials to detect *E. coli* (Pettibone, 1992). The medium was obtained from Difco, USA. The tubes were inoculated with appropriate water sample volume and incubated at 44.5°C for 24hrs. Tubes showing gas production with growth is considered a positive indication of *E. coli* presence , from which several loopfulls were streaked on MacConkey agar plates consisted of (g/liter): Peptone; 17.0, Protease peptone; 3.0, Lactose; 10.0, Bile salts No.3; 1.5, Sodium chloride; 5.0, Agar; 13.5. Neutral red; 0.03. Crystal violet; 0.001 Distilled water; 1.0 liter for testing lactose fermentation ability and suspected colonies showing pink to red color surrounded by red zone (Pettibone, 1992).

Antibiotic sensitivity test for *E. coli* isolates

In vitro susceptibility of *E. coli* isolates against antibiotics was determined by the standard disc diffusion procedure (Bauer *et al*., 1996). 14 commercially prepared antibiotic discs (Oxoid, UK.) (6 mm in diameter) belonging to different groups antibiotics were used, Augmentin, Pipracillin, Imipenem, Levofloxacin, Tobramycin, Gentamycin, Amikacin, Nitrofurantoin, Colistin, Ceftazidime, Naladixic acid, Ciprofloxacin and Amoxycillin. The zones of inhibition were measured, recorded and interpreted according to the Clinical Laboratory Standard institute provided (Croxen *et al.,* 2013).

In this test, the standard Kirby-Bauer disk diffusion method (Bauer *et al*., 1966) was performed in which, 14 antibiotic discs (6 mm in diameter). Four to five similar colonies from overnight growth plate were transferred aseptically in sterile distilled water and vigorously agitated to give a turbidity that matches the 0.5 McFarland standard (approximately 10^8 cfu/ml) according to D'Amato and Hochstein, (1982). Within 15min, sterile cotton swab dipped into the culture suspension was used for inoculating the surface of solidified Mueller-Hinton agar plates (NCCLS/CLSI, 2007). Antibiotic discs were dispensed onto the inoculated plate surface agar and incubated at 37ºC for 24h. The resulted diameters of inhibition zones around the antibiotic discs were measured to nearest whole mm and interpreted according to protocols standardized for the assay of antibiotic compounds as guided by National Committee for Clinical Laboratory Standards "NCCLS". The results were categorized as: R (resistant), I (intermediate sensitive), and S (sensitive) (Hindler, 1998 and NCCLS/CLSI, 2007).

Purification and identification of bacterial isolates.

E. coli colonies obtained from all previously mentioned media were chosen and picked up according to variation in culture characteristics and colony formation then purified by streak-plate method on Nutrient agar medium. Pure isolates were maintained on slants of the same medium at 4ºC for subsequent identification. Identification were carried out automatically by VITECK – 2 System (Biomerieux France). This is an automated new way for identification to save time and efforts. The VITECK – 2 System has certain cards. The Viteck 2 contains software for bacterial strains (Funke *et al.,* 1998). The Viteck system make a rapid correlation of our data base and give use the accrual identification of the stains, where fluorescence is measured every 15 min, and the results of identification are determined.

Results

Bacteriological analysis of csf and fluid. pus, sputum, swab, tip and urine Clinical specimens from Aljala hospital during (January 2017-Decamber 2018) revealed deferent bacteria species on selective media (Table 1) . The results recorded in (Table 1) showed that Frequency of Bacteria detected in Clinical specimens *E.coli* (78.8%) was detected in most Clinical specimens on selective

Table 1: Common bacteria detected from different Clinical specimens on Selective media. TSI (Triple Sugar Iron), MIU (Motility, Indol, and Urea)

Selective media	**Clinical specimens**						
	Csf	**Fluid**	**Pus**	**Sputum**	**Swab**	**Tip**	**Urine**
Blood Agar	*E.coli Streptococcus Neseria*	*E.coli Staphylococcus*	*E.coli Streptococcus*	*E.coli Streptococcus*	*E.coli*	*E.coli Streptococcus*	*E.coli Staphylococcus*
Chocolate agar	*E.coli Neseria*	*E.coli Neseria*	*E.coli Staphylococcus Pseudomonas*	*E.coli Staphylococcus*	*E.coli Staphylococcus Pseudomonas*	*E.coli Staphylococcus Pseudomonas*	*E.coli Staphylococcus Pseudomonas*
Macconky agar	*E.coli*	*E.coli Serritia*	*E.coli Enterobacter*	*E.coli Citrobacter*	*E.coli Pseudomonas*	*E.coli Klebsela*	*E.coli Enterobacter*
CLED Agar	*E.coli Staphylococcus*	*E.coli Staphylococcus Pseudomonas*	*E.coli Staphylococcus Enterobacter*	*E.coli Staphylococcus Enterococcus*	*E.coli Staphylococcus Pseudomonas*	*E.coli Staphylococcus Pseudomonas*	*E.coli Staphylococcus Enterococcus*
S S agar	ND	ND	*Salmonella Shigella*	*Salmonella*	ND	ND	ND
TSI	ND	*E.coli Salmonella*	*E.coli*	*E.coli*	*E.coli Salmonella*	*E.coli*	*E.coli Salmonella*
MIU	ND	E.coli Proteus	*E.coli Klebsela*	*Salmonella*	*E.coli Klebsela*	*E.coli Salmonella*	*E.coli* Proteus
Citrate agar	*E.coli*	*E.coli Staphylococcus*	*Staphylococcus*	*Yersinia*	*E.coli Shigella*	*E.coli Staphylococcus*	*E.coli Staphylococcus*

TSI (Triple Sugar Iron), MIU (Motility, Indol, and Urea)

different media followed by *Staphylococcus* (39.2%), *Pseudomonas(*14.2%)*, Salmonella* (8.9%)*, , Enterobacter* (8.9%) *, Neseria* (7.1%) *, Klebsela* (5.3%) *, Shigella* (5.3%) , Proteus (3.5%) *, Citrobacter* (1.7%) *and Yersinia* (1.7%).

Cultural identification of *E. coli:*

E. coli colonies were round. The round colonies maintained both an entire margin (i.e., continuous, smooth) and a smooth surface. Colonies of *E. coli* show a basic, convex form. Colonies of *E.coli* demonstrated periodic growth pattern, grow in gin waves that result in concentric growth rings in the colony, these rings can be detected under microscopic examination. *E.coli* on blood agar, colonies were big, circular, gray and moist, Beta (B) hemolytic colonies were formed. *E.coli* on MaCconkey agar, colonies are circular, moist, smooth and of entire margin, colonies appear flat and pink, they were lactose fermenting colonies Fig.(1).

Fig.1: Plate gram showing *E. coli* pink colonies on MaCconkey agar media.

Microscopic identification of *E. coli:*

E. coli was Gram negative straight rod, 1-3µx 0.4-0.7µ, arranged singly or in pairs. It was motile by peritrichous flagellae, through some strains are non–motile. Spores were not formed. Capsules and fimbriae were found in some strains.

Identification of *E. coli* by VITEK2 system.

E. coli showing positive reaction with leucine arylamidase, arginine, D-galactose, D-glucose, D-malltose, D-mannose, D-sorbitol, Saccarose/sucrose, D-galactoronate, L-glutamate, acetate,citrate, glucuronate, L-lysine, L-proline, D-raffinose and D-gluconate. As well as *E. coli* showing negative reaction with, Erythritol , Beta-N-Acetyl-glucosaminidase, arbutin,, amygdalin, gentiobiose, lactose, D-cellobiose, gamma-glutamyl-transferase, , D-melibiose, L-sorbose , L-rhamnose, urease, nitrate, L-arabinose and Esculin.

Distribution of *E. coli:*

Data illustrated in Fig. (2) showed the distribution of *E. coli* according to the gender, female was 245 (58%) while the male 177 (42%).

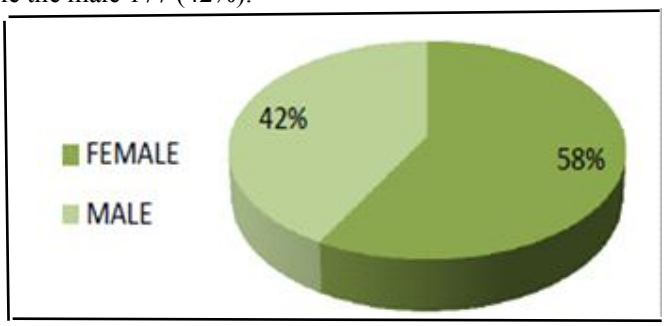

Fig. 2: Distribution of *E. coli* isolates from males and females.

Distribution of *E. coli* clinical isolates according to the age.

The highest age group enrolled in the study was between 21-40 (41%) followed by 41-60 (25%), while the lowest was 1-20 (15%) (Table 3 and Fig. 3).

Table 3: Distribution of *E. coli* clinical isolates according to the age.

Age	1-20	21-40	41-60	62-97
Number	64	171	107	80
%	15%	41%	25%	19%

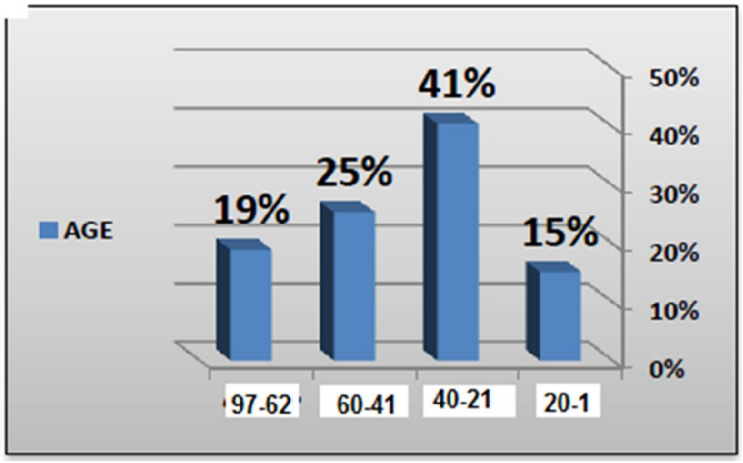

Fig. 3: Distribution of *E. coli* clinical isolates according to the age.

Distribution of *E. coli* growth in the specimens.

The urine was the highest specimen isolate *E. coli* 176 (42%) followed by swab 139 (33%) and tip 84 (20%) while the lowest was recorded in csf 1(0%) (Table 4 and Fig. 4).

Table 4: Distribution of *E. coli* growth in the specimens.

Sample Growth	Swab 33%	Urine 42%	tip 20%	Blood 0%	Pus 4%	Csf 0%	Sputum 0%	Fluid 1%
N	139	176	84	2	15	1	2	3

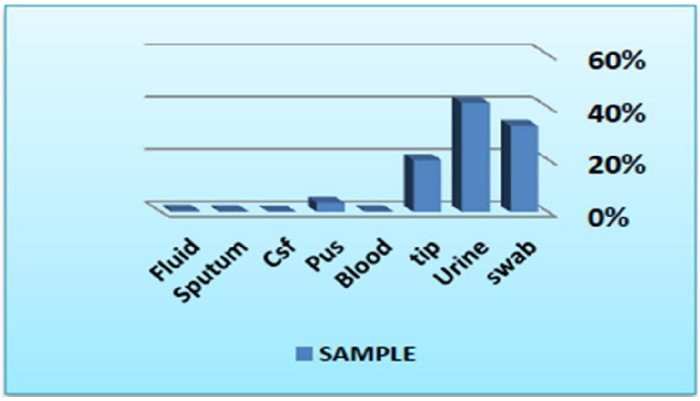

Fig. 4: Distribution of *E. coli* growth in the specimens

Susceptibility and resistant patterns of *E. coli* to the antibiotics.

The high susceptibility of *E. coli* was record toward Amikacin 330 (78%) followed by Impienem 320 (76%) and Colistin 244 (58%) while high resistant was observed toward Aug 238 (56%) followed by Septrin 197(47%) and Ciprofloxacin 140 (33%) (Table 5 and Fig. 5)

Table 5: Susceptibility and resistant patterns of *E. coli* to the antibiotics

***E. coli* isolates**		**Antibiotics**													
		Amk	**ct**	**Aug**	**Cip**	**F**	**Cft**	**NA**	**pip**	**GN**	**PB**	**Imp**	**SXT**	**Levo**	**Tob**
E.coli-1	Growth rate	47	2	18	11	15	1	4	4	62	0	3	7	19	13
	% Inhibition	11	0	4	3	4	0	1	1	15	0	1	2	5	3
E.coli-2	Growth rate	330	244	63	249	181	63	93	89	198	53	320	116	223	20
	% Inhibition	78	58	15	59	43	15	22	21	47	13	76	27	53	5
E.coli-3	Growth rate	35	11	238	140	19	68	46	50	129	2	16	197	113	63
	% Inhibition	8	3	56	33	5	16	11	12	31	0	4	47	27	15
E.coli-4	Growth rate	10	165	103	22	207	290	279	279	33	367	83	102	67	326
	% Inhibition	2	39	24	5	49	69	66	66	8%	87	20	24	16	77

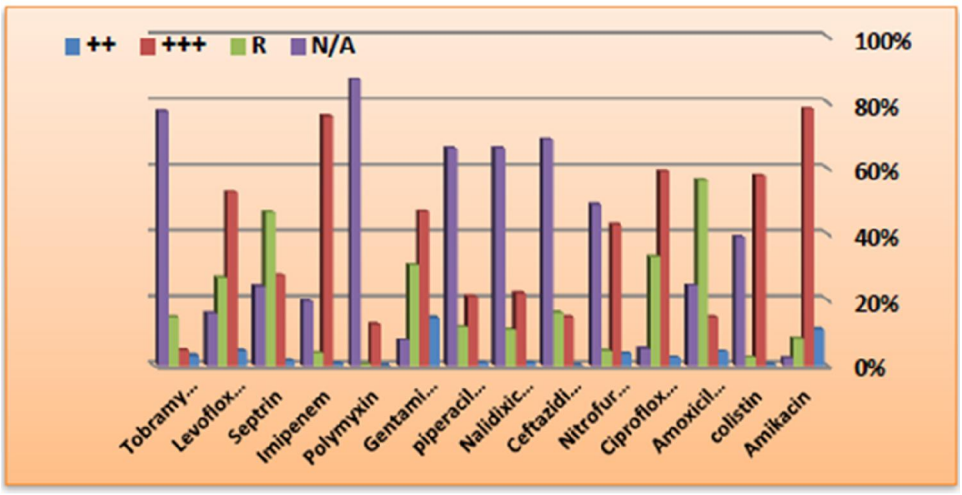

Fig. 5: Susceptibility and resistant patterns of *E. coli* to the antibiotics.

Discussion

Antibiotic resistance bacteria are majorly a problem of public health worry. The aim of this study to isolate and identify antibiotic resistance bacteria infected csf, fluid, pus, sputum swab, tip and urine 422 clinical specimens collected from the clinical locations at Al- jala hospital- Benghazi at Al- jala hospital- Benghazi. The clinical specimens were analyzed using standard microbiological. Common bacteria detected from different Clinical specimens on Selective media. TSI (Triple Sugar Iron), MIU (Motility, Indol, and Urea) *E. coli* followed by *Staphylococcus* and *Pseudomonas*. Forty patients showing different types of infections were included, 92 samples were collected which included blood, urine, swab, sputum, pus, and endotracheal tube (ETT) samples were found positive, i.e. showed the growth of microorganisms. (*Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., *Staphylococcus aureus*, *Enterococcus*, *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., and *Candida* spp (Shamim *et al.,* 2018). *E. coli* isolates were dominant detected in different Clinical specimens on selective media (Coia *et al.,* 2017). The isolated *E. coli* was microbiological and biochemical identified by Vitek 2 system (Pettibone, 1992 and Funke *et al.,* 1998). The clinical specimens of different infection sources, 422 specimens were isolated and identified as *E. coli*. *Escherichia coli* from 422 clinical specimens of csf, fluid, pus, sputum swab, tip and urine collected from the clinical locations. In our study group there were mostly women, is similar with the data from literature (Ioana *et al.,* 2010). Most strains of *E. coli* were isolated from urine followed by swab. Is contrast to study that conduct the most strains of *E. coli* were isolated from purulent skin ulcers secretions (Ioana *et al.,* 2010). It was determined antibiotic susceptibility pattern (Ioana *et al.,* 2010). The high susceptibility of *E. coli* was record toward Amikacin 330 (78%) followed by Impienem 320 (76%), that similar to study recorded (75%) sensitivity to amikacin, (52.3%) to imipenem (Ioana *et al.,* 2017, Gums, 2005 and Junie, 2004) and contrast to study was recorded the highest sensitivity was to Carbapenems (93%).18 *E. coli* isolates were resistant to Aug, is similar to study conduct *E. coli* isolates were resistant to Augmentin. (Reynolds *et al.,* 2011). Effective hygiene must be encouraged and unselective usage of antibiotics must be avoided.

Conclusion

Wrong usage of Antibiotic drugs which generates increasing of *E. coli* infections in an unclean environment of some hospitals. In this study, we found the disc diffusion to be a reliable, easy and inexpensive method for testing the susceptibility of *E. coli* to: toward Amikacin, Impienem and Colistin.

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