Increased multi-drug resistant *Escherichia coli* from hospitals in Khartoum state, Sudan

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Abstract

Background: Multidrug-resistant *Escherichia coli* (MDR *E. coli*) has become a major public health concern in Sudan and many countries, causing failure in treatment with consequent huge health burden.

Objectives: To determine the prevalence and susceptibility of MDR *E. coli* isolated from patients in hospitals at Khartoum State.

Methods: Between May to August 2011, *E. coli* (n = 232) isolated from clinical specimens, identified, tested their antimicrobials susceptibility and screened for extend spectrum â-lactamase production as per standard methods.

Results: Of the 232 *E. coli* isolates, the majority were from urine (65.1%). MDR *E. coli* were present in 214 (92.2%). Of these, the resistance rates were recorded to: amoxicillin 97.7%, cefuroxime 92.5%, trimethoprim-sulfamethoxazole 88.3%, tetracycline 77.1%, nalidixic acid 72%, ceftriaxone 64%, ciprofloxacin 58.4%, ofloxacin 55.1%, amoxicillin-clavulanate 50.4%, ceftazidime, gentamicin 35% each, nitrofurantoin 22.4%, chloramphenicol, tobramicin 18.2% each and amikacin 1.9%. Overall MDR *E. coli*, 53.3% were resistant to > 7 antimicrobial agents and ESBL was detected in 32.7%. Isolates from males were more resistant than those from females ($p < 0.05$).

Conclusions: Drug-resistance surveillance and epidemiological analysis of patient data is need periodically and can be informative for appropriate management of antimicrobial resistance.

Keywords: Multi-drug resistance, *E. coli*, Sudan

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Introduction

Escherichia coli (*E. coli*) is a common pathogen linked with community-associated as well as nosocomial infections[1,2]. In the last few years, the emergence and wide dissemination of *E. coli* strains showing resistance to broad-spectrum of antimicrobial agents has been reported[1,3,6]. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria[3-7]. According to the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), multi-drug resistant (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories[4]. MDR bacteria are the principal cause of failure in the treatment of infectious diseases, resulting in increases in the term and magnitude of morbidity, higher rates of mortality, and a greater health cost burden[9,10]. However, recently researchers have been investigated the activity of non-antimicrobial agents such as proanthocyanidin to prevent MDR bacterial infections[29].

Multi-drug resistant *E. coli* are widely distributed in hospitals and are increasingly being isolated from community. Thus, it is urgent need to find out new antimicrobial agents[8,10,11]. However, new families of antimicrobial agents have a short life expectancy[11]. Several monitoring programmes have been initiated to generate baseline data about the prevalence of MDR in different bacterial species, including *E. coli*[8,12]. Many studies from Europe[7] and USA[6,13] have investigated MDR among *E. coli* isolates. Most bacterial isolates from Asian and African countries have shown high MDR rates[14-18]. In Sudan, limited data are currently available on the prevalence

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of MDR amongst significant bacterial isolates[19,20]. The aims of this study were to determine the prevalence and antimicrobials susceptibility of MDR *E. coli* collected from clinical specimens of patients in different hospitals in Khartoum State. It also, investigates the possible differences in antimicrobial resistance of *E. coli* isolated from a various type of clinical specimens in relation to the patient sex, age and settings

Methods

Isolates

Between May to August 2011, *E. coli* (n = 232) were isolated from clinical specimens of patients at different teaching hospitals in Khartoum State, Sudan. The participating hospitals were: Khartoum Teaching Hospital, Khartoum North Educational Hospital, National Health Laboratory, Omdurman Teaching Hospital, Soba University Hospital and Turkish Teaching Hospital.

E. coli were isolated from patient clinical specimens including urine, wound pus, high vaginal swabs, Ear discharges, blood, stool, semen and miscellaneous body fluids after following conventional procedures[21]. Significant bacterial growth was included in this study and they were identified on the basis of cultural characteristics, gram stain and conventional biochemical tests[22] then confirmed by API 20E identification system (Biomerieux Marcy-I'Etoile, France). When confirmed as *E. coli*, the isolates were preserved at -70 ºC in tryptic soy broth containing 20% sterile glycerol. The database of each specimen source and patient sex, age and setting were recorded.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *E. coli* isolates was performed on Muller-Hinton agar plate (Oxoid, Basingstoke England) by the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute- (CLSI)[23]. The antimicrobial agents which were tested from different categories (Table 1) including: amikacin (30µg), amoxicillin (10µg), amoxicillin-clavulanate (30µg), ceftazidime (30µg), ceftriaxone (30ìg) cefuroxime (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µµg), nalidixic acid (30µg), nitrofurantoin (50µg), ofloxacin (5µg), tetracycline (30µg), tobramicin (10µg) and trimethoprim-sulfamethoxazole (25µg) (Oxoid, England). *E. coli* ATCC 25922 was used as control strains and was tested each time when susceptibility testing was performed. Test results were only validated in the cases where inhibition zone diameters of the control strains were within performance ranges in accordance to guidelines[23]. Resistant and intermediate results were considered as non-susceptible[4]. MDR *E. coli* was defined as non-susceptibility to at least one agent in three or more antimicrobial categories[4].

Extend spectrum â-lactamase production

All *E. coli* isolates recognizing reduced susceptibility to ceftazidime and ceftriaxone they were screened for ESBLs production by the double-disk synergy test according to CLSI recommendations[23].

In this test, the organism is swabbed onto a Mueller-Hinton agar plate. A susceptibility disk containing amoxicillin-clavulanate is placed in the center of the plate, and disks containing ceftazidime and cefotaxime are placed 30 mm (center to center) from the amoxicillin-clavulanate disk. A clear extension of the edge of the inhibition zone of cephalosporin towards amoxicillin-clavulanate disk is interpreted as positive for ESBL production[24].

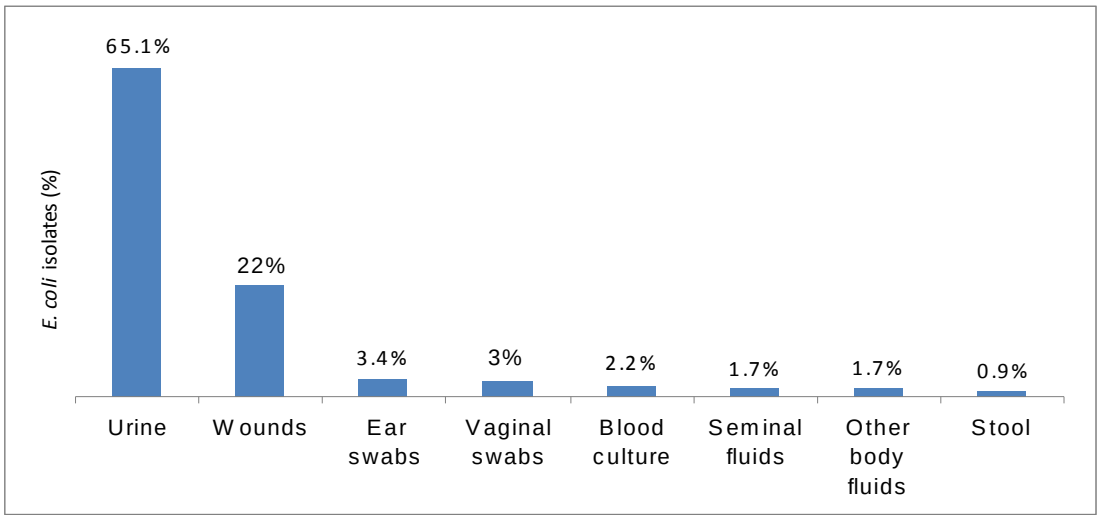
Statistical analysis

All outcome data were analyzed using Statistical Package for Social Sciences (SPSS; Version10.0). The differences between resistance patterns of *E. coli* strains were determined using Independent samples T-Test and One-Way Analysis of Variance (ANOVA). All p-values were based on 2-tailed tests of significance where $p < 0.05$ is considered statistically significant.

Results

The prevalence of *E. coli* recovered from various clinical specimens (232) collected from different hospitals in Khartoum State is shown in Figure 1. Urine (65.1%) and wound (22%) specimens represented the majority of specimens. The isolates were obtained from all aged groups: 138 (59.5%) were females and 94 (40.5%) were males. Of the total number of isolates, 180 (77.6 %) were obtained from adult patients.

Figure 1: Prevalence of *E. coli* (n = 232) recovered from various clinical specimens collected from hospitals in Khartoum State



Antimicrobial resistance rates and multidrug-resistant phenotypes

Of the 232 isolates tested, MDR *E. coli* were present in 214 (92.2%). Of these 32.7 % were found to be ESBL producer. Antimicrobial resistance patterns of the 214 MDR *E. coli* isolates against 15 antimicrobial agents is shown in table 1. High resistance rates were observed to amoxicillin (97.7%), cefuroxime (92.5%), trimethoprim-sulfamethoxazole (88.3%), tetracycline (77.1%), nalidixic acid (72%) and ceftriaxone (64%). Moderate resistance rates were observed to ciprofloxacin (58.4%), ofloxacin (55.1%), amoxicillin-clavulanate (51.4%), ceftazidime and gentamicin (35% each). Lower resistance rates were observed for nitrofurantoin (22.4%), chloramphenicol and tobramicin (18.2% each). Resistance to amikacin was uncommon (1.9%).

Table 1: Antimicrobial resistance pattern among *E. coli* (n = 214) isolated from different hospitals in Khartoum State, Sudan

Antimicrobial and category*	Hospital						Overall agent antimicrobial resistance (%)
	Khartoum (n= 68)	KNH (n=32)	NHL (n=11)	Omdurman (n= 35)	Soba (n= 42)	Turkish (n=26)	
Penicillins							
Amoxicillin	98.5	96.9	100	97.1	97.6	96.2	97.7
Penicillins + â-lactamase inhibitors							
Amoxicillin-CA	47.1	56.3	36.4	57.1	52.4	53.8	51.4
Nom-extend spectrum cephalosporins; 1st and 2nd generation							
Cefuroxime	92.6	93.8	90.9	100	88.1	88.5	92.5
Extend-spectrum cephalosporins; 3rd generation							
Ceftriaxone	73.5	59.4	45.5	71.4	59.5	50	64
Ceftazidime	51.5	18.8	18.2	40	23.8	30.8	35
Quinolones							
Ciprofloxacin	57.4	68.8	81.8	51.4	50	61.5	58.4
Nalidixic acid	70.6	81.3	90.9	62.9	69.0	73.1	72
Ofloxacin	55.9	62.5	63.6	51.4	45.2	61.5	55.1
Folate pathway inhibitors							
SXT	92.6	81.3	90.9	94.3	83.3	84.6	88.3
Tetracyclines							
Tetracycline	82.4	68.8	72.7	91.4	64.3	76.9	77.1

Continuation of table 1

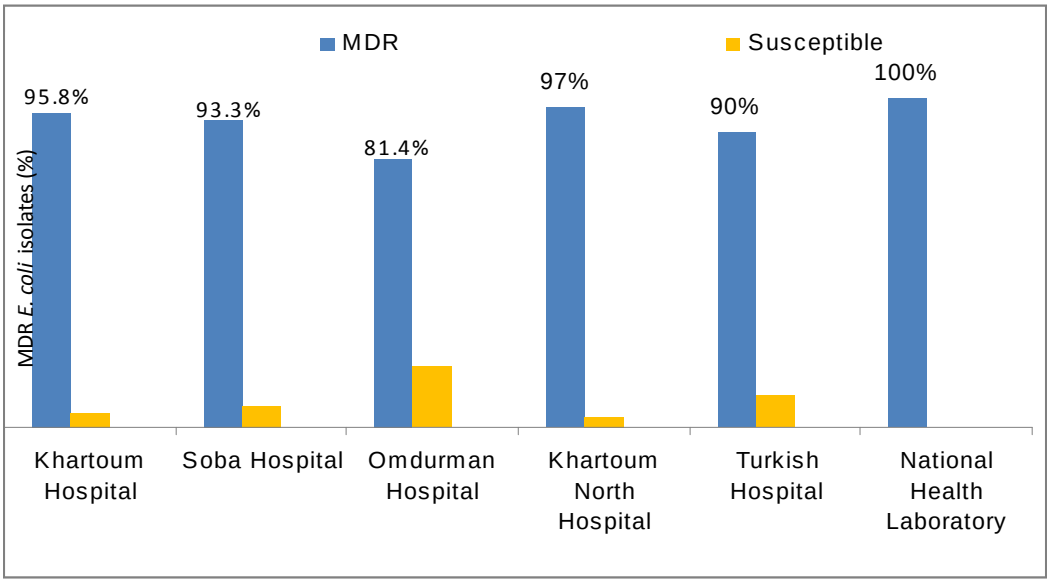
Antimicrobial and category*	Hospital						Overall agent antimicrobial resistance (%)
	Khartoum (n= 68)	KNH (n=32)	NHL (n=11)	Omdurman (n= 35)	Soba (n= 42)	Turkish (n=26)	
Aminoglycosides							
Amikacin	1.5	3.1	0	0	4.8	0	1.9
Gentamicin	35.3	31.3	9.1	37.1	40.5	38.5	35
Tobramicin	19.1	9.4	36.4	17.1	11.9	30.8	18.2
Phenicols							
Chloramphenicol	32.4	18.8	0	17.1	4.8	11.5	18.2
Nitrofurans							
Nitrofurantoin	26.5	18.8	27.3	28.6	16.7	15.4	22.4

* Adapted from Magiorakos *et al.* (2011).
KNH = Khartoum North Hospital; NHL = National Health Laboratory; CA = Clavulanate; SXT = Trimethoprim-sulfamethoxazole

Frequency of antimicrobial resistance pattern of *E. coli* by hospitals

The frequencies of MDR *E. coli* isolated from different hospitals in Sudan are shown in Figure 2. The resistance rates of isolates to ceftazidime, ceftriaxone and chloramphenicol were significantly higher ($p < 0.05$) in Khartoum hospital than other hospitals. The resistance rates of isolates to ciprofloxacin and ofloxacin were found significantly higher in Khartoum and Khartoum North than Soba and Omdurman hospitals ($p < 0.05$) (table 1). But the resistance rates for nitrofurantoin, tetracycline, trimethoprim-sulfamethoxazole, were found significantly higher in isolates from Khartoum and Omdurman hospitals than isolates from Soba and Khartoum North hospitals ($p < 0.05$). The resistance rates of isolates to gentamicin were found high in Soba hospital but low resistance rates were observed to chloramphenicol (table 1).

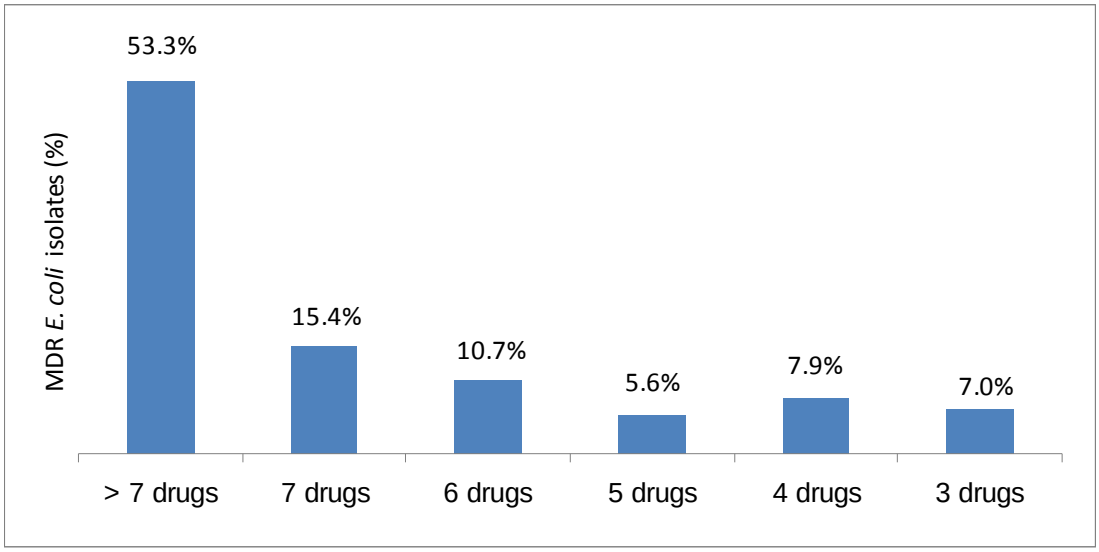
Figure 2: Distribution of MDR *E. coli* (n = 214) isolated from different hospitals in Khartoum state



Frequency of MDR *E. coli* according to the number of drug resistance

The frequency of MDR to 3, 4, 5, 6, 7 and more than 7 of total 15 antimicrobial agents were 15 (7.0%), 17 (7.9%), 13 (5.6%), 23 (10.7%), 33 (15.4%) and 114 (53.3%), respectively. Of the 92.2% MDR isolates, the most prevalent patterns were resistance to more than 7 (114; 53.3%), followed by 7 (33; 15.4%) and 6 (23; 10.7%) of antimicrobial agents (figure. 3).

Figure 3: Frequency of MDR *E. coli* (n = 214) according to its resistance to three or more antimicrobial agents



Frequency of antimicrobial resistance pattern of *E. coli* by sex and age

Table 2 shows the resistant pattern of the 214 MDR *E. coli* isolated from males and females. Isolates from males were showed higher resistance rates than those from females to ceftazidime (p = 0.023), ceftriaxone (p = 0.004), chloramphenicol (p = 0.005), nalidixic acid (p = 0.002), ofloxacin (p = 0.024), tetracycline (p =0.006) and trimethoprim-sulfamethoxazole (p = 0.02). In contrast, resistance rates to nalidixic acid and ofloxacin were higher in females than males isolates (table 2).

Distribution of MDR *E. coli* according to age showed no statistical differences between adults and children were observed to the most antimicrobial agents. However, there were significant differences observed in two antimicrobial agents ciprofloxacin (p = 0.02) was higher in adults and chloramphenicol (p = 0.047) was higher in children (table 2).

Table 2: Antimicrobial resistance (%) among *E. coli* (n = 214) isolated from different hospitals in Khartoum state in relation to patient's sex and age

Antimicrobial agent	Sex			Age		
	Male (n= 89)	Female (n= 125)	p. value	Adult (n= 168)	Child (n=46)	p. value
Amikacin	1.1	2.4	0.499	1.8	2.2	0.864
Amoxicillin	96.6	98.4	0.400	97	100	0.238
Amoxicillin-clavulanate	48.3	53.6	0.448	51.8	50	0.831
Ceftazidime	43.8	28.8	0.023	35.7	32.6	0.697
Ceftriaxone	75.3	56	0.004	63.7	65.5	0.849
Cefuroxime	95.5	90.4	0.163	93.5	89.1	0.326
Chloramphenicol	27	12	0.005	15.5	28.3	0.047
Ciprofloxacin	50.6	64	0.050	62.5	43.5	0.020
Gentamicin	38.2	32.8	0.417	34.5	37	0.761
Nalidixic acid	60.7	80	0.002	75	60.9	0.059
Nitrofurantoin	24.7	20.8	0.500	22.6	21.7	0.900
Ofloxacin	46.1	61.6	0.024	58.3	43.5	0.073
Tetracycline	86.5	70.4	0.006	77.4	76.1	0.854
Tobramicin	21.3	16	0.320	19	15.2	0.553
Trimethoprim-sulfamethoxazole	94.4	84	0.020	87.5	91.3	0.479

Discussion

The present study showed that the prevalence of MDR among *E. coli* isolates was higher (92.2%) in comparison to previous study carried out in Sudan[19]. These authors recorded 58% MDR *E. coli* isolated from urinary tract infections in the year 2000. In this study, the prevalence of MDR *E. coli* isolates is similar to those reported in south America[3], but relatively higher than those reported in neighboring countries such as 87% in Egypt[15] and 74.6% in Ethiopia[14] and other countries as 83.9% in Nigeria[16] and 74% in Saudi Arabia[17]. Furthermore, our findings are much higher compared to those reported in Europe[1] and USA[6].

In this study, there are high resistance rates of MDR *E. coli* isolates to the first-line oral antimicrobial agents such as amoxicillin, cefuroxime, trimethoprim-sulfamethoxazole, tetracycline nalidixic acid and amoxicillin-clavulanate These findings represent alarming increased rates in resistant *E. coli* are comparable to other studies in Sudan[19] and elsewhere[3,6,25].

Resistance to fluoroquinolones varies geographically and is an emerging problem in both developed and developing countries.[13,28]. In the present study, MDR *E. coli* isolates showed relatively high resistance rates to ofloxacin and ciprofloxacin. This has been hypothesized to be related to the inappropriate use of fluoroquinolones for humans[2]. Also, prolonged use of low dose of the more potent fluoroquinolones such as ciprofloxacin has been shown to be the most significant risk factor for acquisition of resistance[27].

Whilst the third-generation cephalosporins such as ceftriaxone and ceftazidime have been used to treat gram-negative bacterial infections of various body sites[5,25], the current study showed high levels of resistance to second and third generation cephalosporins. A possible explanation for the high resistance found might be the presence of ESBL in these strains [18]. Since ESBL mediated resistance to wide range of antimicrobial classes [8,18], it is important that routine screening of ESBL in clinical isolates is carried out to prevent widespread of resistant isolates in our hospitals.

Like studies elsewhere [7,15,25], our MDR *E. coli* isolates were found to be effective against aminoglycoside agents. Amikacin appears to have wider range of activity than tobramicin, gentamicin and other tested antimicrobial agents. The explanation for amikacin is probably the fact that these are very powerful drugs used only in hospital settings and not as first-line therapy. Therefore, they have lower selective pressure due to their restricted use[25].

The emergence of *E. coli* isolates with different MDR phenotypes, involving co-resistance to three or more unrelated families of antimicrobial agents, has been previously reported by others and is considered a serious health concern[1,3,6]. Similar to reports by others [3,7,16], the current study expressed high resistance rates to different classes of antimicrobial agents. Almost our MDR *E. coli* isolates were found to be multi-resistant to the commonly used antimicrobials agents of amoxicillin, cefuroxime, trimethoprim-sulfamethoxazole tetracycline, and nalidixic acid. These resistance profiles were common and could be accounted for by a number of known acquired resistance genes[3]. Since proanthocyanidin is a non-antimicrobial agents used for the prevention of urinary tract infection caused by susceptible *E. coli* strains via inhibiting P-fimbriated adhesion to uroepithelial cells[30]. Recently, Gupta *et al.*[29] have been observed that proanthocyanidin prevent also adhesion of most MDR *E. coli* strains to the uroepithelial cells.

In the present study, MDR *E. coli* from Khartoum hospital had high resistance rates to most of the antimicrobial agents. Also, our report showed a variable difference in antimicrobial resistance of MDR *E. coli* between each hospital in Khartoum State. MDR may vary according to geographical regions. Although previous use of antimicrobials may be involved in the regional differences of antimicrobial resistance, consider other factors such as the patients' characteristics or sampling bias and the social and geological factors that attribute to this variation of MDR as previously described[26]. Large-scale, local studies, hospital and departmental data, therefore, are required to understand drug resistance in each setting.

In the current study, MDR *E. coli* isolates from males were more likely to have resistance to antimicrobial agents than those from females. Similar findings have been described worldwide[6,7,13,25]. The resistance pattern was somewhat affected in gender, but that definitely depended on the site of infection[25] and clinical parameters of the population investigated[26].

No statistical differences ($p = 0.249$) were found in antimicrobial resistance between adults and children. Similar results were reported by Boyd *et al.* (2008). However, it is important to emphasize on the high prevalence of fluoroquinolones resistance in isolates

from children which is not recommended for treatment of pediatric patients.

Conclusion

This study concluded that MDR *E. coli* is escalating in Khartoum State (92.2%) compared to previous surveys (58%). Multiple resistances to antimicrobial drugs among *E. coli* isolates complicate therapeutic management of infections. Drug-resistance surveillance and epidemiological analysis of patient data is needed periodically and can be informative for appropriate management of antimicrobial resistance. Understanding the molecular basis of resistance acquisition and transmission can contribute to the development of new strategies to combat this phenomenon.

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References

1- Oteo J, Lázaro E, de Abajo FJ, Baquero F, Campos J. Spanish members of EARSS. Antimicrobial-resistant invasive *Escherichia coli*, Spain. *Emerg Infect Dis* 2005; 11:546-553.

2- Drago L, Nicola L, Mattina R, Vecchi, ED. In vitro lselection of resistance in *Escherichia coli* and Klebsiella spp. at in vivo fluoroquinolone concentrations. *BMC Microbiol* 2010; 10:119.

3- Bartoloni A, Pallecchi L, Benedetti M. *et al.* Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. *Emerg Infect Dis* 2006; 12:907-913.

4- Magiorakos AP, Srinivasan A, Carey RB. *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2011 May 7. doi: 10.1111/j.1469-0691.2011.03570.x.

5- Bilal NE and Gedebou M. Clinical and community strains of *Klebsiella pneumoniae*: multiple and increasing rates of antibiotic resistance in Abha, Saudi Arabia. *Br J Biomed Sci* 2000; 57:185-191.

6- Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA. Multidrug-resistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States. *Antimicrob Agents Chemother* 2001; 45:1402-14026.

7- Oteo J, Campos J, Baquero F. Spanish members of the European Antimicrobial Resistance Surveillance System. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System (2001). *J Antimicrob Chemother* 2002; 50:945-952.

8- Peralta G, Sánchez MB, Garrido JC. Impact of antibiotic resistance and of adequate empirical antibiotic treatment in the prognosis of patients with *Escherichia coli* bacteraemia. *J Antimicrob Chemother* 2007; 60:855-863.

9- Howard DH, Scott RD 2nd, Packard R, Jones D. The Global Impact of Drug Resistance. *Clin Infect Dis* 2003; 36(Suppl 1):S4-1

10- Coates A, Hu Y, Bax R, Page C. The future challenges facing the development of new antimicrobial drugs. *Nat Rev Drug Discov* 2002; 1:895-910.

11- Rahman S, Parvez AK, Islam R, Khan MH. Antibacterial activity of natural spices on multiple drug resistant *Escherichia coli* isolated from drinking water, Bangladesh. *Ann Clin Microbiol Antimicrob* 2011; 10:10.

12- Guerra B, Junker E, Schroeter A, Helmuth R, Guth BEC, Beutin L. Phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* O111 isolates. *J Antimicrob Chemother* 2006; 57:1210-1214.

13- Boyd LB, Atmar R, Randall GL, Hamill, RJ, Steffen D, Zechiedrich L. Increased fluoroquinolone resistance with time in *Escherichia coli* from >17,000 patients at a large county hospital as function of culture site, age, sex, and location. *BMC Infect Dis* 2008; 8:4.

14- Kibret M, Abera B. Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia *Afr Health Sci* 2011; 11(S1): S40 - S45

15- Salem MM, Muharram M, Alhosiny IM. Distribution of Classes 1 and 2 Integrons among Multi Drug Resistant *E. coli* Isolated from Hospitalized Patients with Urinary Tract Infection in Cairo, Egypt. *Australian Journal of Basic and Applied Sciences* 2010; 4:398-407.

16- Ngwai YB, Akpotu MO, Obidake RE, Sounyo AA, Onanuga A, Origbo SO. Antimicrobial susceptibility of *Escherichia coli* and other coliforms isolated from urine of asymptomatic

students in Bayelsa State, Nigeria. *African Journal of Microbiology Research* 2010; 5:184-191.

17- Bilal NE, Gedebou M, Al-Mohayia MH. Gram-negative bacilli from hospital and non-hospital personnel: pharyngeal carriage, multi-drug resistance and extended-spectrum â lactamase in Abha, Saudi Arabia. *Biomedical Research* 2001; 12: 251-258.

18- Kader AA, Kumar AK. Prevalence of extended-spectrum â-lactamase among multidrug resistant Gram-negative isolates from a general hospital in Saudi Arabia. *Saudi Med J.* 2004; 25: 570-574.

19- Ahmed AA, Osman H, Mansour AM. et al. Antimicrobial agents' resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. *Am J Trop Med Hyg* 2000; 63:259-263.

20- Hamdan HZ, Ziad AHZ, Ali SK, Adam I. Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. *Ann Clin Microbiol Antimicrob* 2011; 10:2.

21- Thomson Jr RB, Miller JM. Specimen collection, transport, and processing: bacteriology. In: *Manual of Clinical Microbiology*. 8th edition, edited by Murray PR, Baron EJ, Pfaller MA, Tenover, FC and Yolken RH., Washington, DC: American Society for Microbiology 2003; 286-330.

22- Farmer JJ 3rd. *Enterobacteriaceae*: Introduction and Identification. In: *Manual of Clinical Microbiology*. 8th edition, edited by Murray PR, Baron EJ, Pfaller MA, Tenover, FC and Yolken RH., Washington, DC: American Society for Microbiology 2003; 636-653.

23- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; twenty first Informational supplement. CLSI document M100-S21, Wayne, Pa: Clinical and Laboratory Standards Institute, 2011; 31(1).

24- Jarlier V, Nicolas MH Fournier G Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10:867-878.

25- Sahuquillo-Arce JM, Selva M, Perpin˜a´n H. *et al.* Antimicrobial Resistance in More than 100,000 *Escherichi coli* Isolates According to Culture Site and Patient Age, Gender, and Location. *Antimicrob Agents Chemother* 2011; 55:1222-1228.

26- Lee G, Cho YH, Shim BS, Lee SD. Risk Factors for Antimicrobial Resistance among the *Escherichia coli* Strains Isolated from Korean Patients with Acute Uncomplicated Cystitis: A Prospective and Nationwide Study. *J Korean Med Sci* 2010; 25:1205-1209.

27- Chenia, HY, Pillay B, Pillay D. Analysis of the mechanisms of fluoroquinolone resistance in urinary tract pathogens. *J Antimicrob Chemother* 2006; 58:1274-1278.

28-Namboodiri SS, Opintan JA, Lijek RS, Newman MJ, Okeke IN. Quinolone resistance in *Escherichia coli* from Accra, Ghana. *BMC Microbiol* 2011; 11:44.

29- Gupta A, Dwivedi M, Mahdi AA, Nagana Gowda GA, Khetrapal CL, Bhandari M. Inhibition of adherence of multi-drug resistant *E. coli* by proanthocyanidin. *Urol Res* 2012; 402:143-150.

30- Howell AB, Botto H, Combescure C, *et al.* Dosage effect on uropathogenic *Escherichia coli* anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: a multicentric randomized double blind study. *BMC Infect Dis.* 2010; 10:94.