ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Mass gatherings pose a threat to the spread of antimicrobial resistant *E. coli* strains.

Kamelia M Osman [1]*, Mahmoud D ElHariri[1], Ghada S AbdElHamed[2]

1.Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Cairo, Egypt
2.Department of Serology, Animal Health Research Institute, Dokki, Egypt

Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 25 March 2014
Published Online: April 2014

Key words:
Mass gatherings, *E. coli*, antibiotic resistance, human, poultry

Corresponding Author

Kamelia M Osman

Abstract

The results of antimicrobial sensitivity test of *E. coli* recovered from human samples indicated that 29/29 strains (100%) were resistant to tetracycline, naldixic acid, ampicillin, trimethoprim, neomycin, oxytetracycline, sulpha-methoxazole trimethoprim, erythromycin and 25/29 strains (86.2%) were sensitive to colistin sulfate. On the other hand, 57/57 (100%) poultry strains were resistant to oxytetracycline and streptomycin, 7/57 (12.3%) were resistant to gentamycin while 100% were sensitive to colistin sulfate. The results also record a severe diversity in the antimicrobial resistance profiles of *E. coli* isolates collected from human and poultry

Introduction

Features of Mass gatherings (MGs) in the form of crowding, inadequate water and sanitation, poor access to health services, characteristic of sudden population displacement, increase the risk of communicable disease transmission (Toole, 1997). The participants constitute variations in their lifestyle, culture, ethnicity and socio-economic status. The participants had their meals in fast food restaurants and from vendors. Mass gatherings pose the usual concerns with people in close contact, such as bacterial gastrointestinal infections. There are also more remote concerns, such as the introduction of diseases from distant locations, such as multiresistant low-dose enteric infections that show person-to-person spread. Drug-resistant microbes of all kinds can move among people and animals, from one country to another—without notice. National and international participants allows subjects to acquire the uniquely resistant strains in a particular locale and to transport these strains back and forth into the area and thus contribute to a state of dissemination throughout Egypt in addition to the resistant strains that accompany international participants who serve as disseminating carriers to these resistant strains. The problem of antimicrobial resistance knows no boundaries. The risk of subsequent spread of *E. coli* resistant strains at MGs, and when participants return to their homes becomes inevitable. The dissemination of multidrug-resistant *E. coli* could have a devastating effect on the attendees at the MGs. Several reports have suggested the use of tetracyclines, sulfa drugs, cephalosporins, and penicillins to be a major factor in the emergence and dissemination of antimicrobial-resistant *E. coli* (Doyle et al., 2013). However, a relative paucity of information exists regarding antimicrobial resistance in *E. coli* from nonhospital sources, especially those from animal sources (Woodford et al., 2014). Therefore, assessing potential public health risks during the MGs and the capacity of the existing surveillance and response structures to detect and control them is therefore a matter of concern.

Consequently, the current study was an endeavor to obtain a generalized understanding of the prevalence and the epidemiological diversity of the antimicrobial-resistance of 86 *E. coli* isolates from human (29) diagnostic samples gathered during the MGs between January 2011 and July 2013 in Tahreer Square and from poultry (57) in the vicinity of Kasr AlAini Hospital area.

Materials and Methods

Isolation of *E. coli* strains from fresh faeces and urine

Rectal swabs were directly plated on Chromogenic agar (BBL CHROMagar O157 CHROM, Becton Dickinson, USA) and incubated at 37°C overnight (O157:H7 appear as mauve cells, while non-O157 VTEC typically present as either pink or blue colonies). Colonies typical of *E. coli* were streaked onto eosin methylene blue (EMB) agar (Becton Dickinson, Sparks, MD, USA) which were incubated at 37°C for 18 to 20 h. Colonies with a metallic sheen on EMB agar were picked and streaked onto MacConkey (Becton Dickinson) and EMB agar (Merck). After 24 to 48 h of incubation (37°C), gram-negative microorganisms were randomly picked from each MacConkey and EMB agar plates and the isolates were tested by the indole, methyl red, Voges–Proskauer and Simmons citrate (IMVic) tests as putatively *E. coli*. The strains were subsequently characterized by the biochemical API 20E test system (Biomerieux) according to the manufacturer's instructions. The *E. coli* isolates were stored in 5% glycerol-supplemented broth at -70°C until use.

Urine culture was done using chromogenic agar (BBL CHROMagar O157 (CHROM, Becton Dickinson, USA), followed by conventional identification. *E. coli* isolates were stored in 5% glycerol in Trypticase soy broth (TSB, Cat. No. 257107 Becton, Dickinson and Company, USA) at -70°C until use.

The study area of Kasr AlAini was chosen because it receives patients from both rural farming and urbanized areas and so offer a rural–urban contrast in *E. coli* disease in addition to the patients that were received from AlTahreer square MG during the period between January 2011 and July 2013 (Fig. 1).

Analysis of antimicrobial susceptibility

Antimicrobials and range of concentrations tested are given in Table 1. Multidrug resistant isolates are defined as those resistant to more than three classes of antibiotics. The 86 purified isolates were cultured in trypticase-soy broth (TSB) supplemented with 0.6% yeast extract, and transferred to Mueller–Hinton agar (Oxoid). The plates were incubated at 37^{o}C for 48 h. After incubation, the diameter of the halos was measured. Resistance was determined by measurement of inhibition of growth around the antimicrobial disk according to the zone diameter interpretative standards of CLSI (2010) or according to the antimicrobials manufacturers' instructions. Susceptibility and resistance were determined according to the interpretation criteria to *E. coli* standard (ATCC No. 25922) established by CLSI.

PCR detection for *E. coli*

Freshly grown typical *E. coli* colonies were boiled in 400 µl of 1 X Tris-EDTA buffer (pH 8.0) (approximately 10^8 cells/ml) boiled for 10 min and centrifuged at 14,000 rpm for 10 min to remove denatured proteins and bacterial membranes. *E. coli* was distinguished on the basis of the *uid*A gene encoding b-glucuronidase specific for *E. coli* (Heininger et al., 1999) that can be revealed by PCR (forward primer 5' ATC ACC GTG GTG ACG CATGTC GC 3 and reverse primer 5' CAC CAC GAT GCC ATG TTC ATCTGC 3') to amplify the 468-bp fragment. A stock buffered solution containing (250 µL) 10X PCR buffer, 100 µL of 125 mM Mg Cl $_2$, 12.5 µL of each dNTPs (ATP, TTP, GTP and CTP) at a concentration of 10 mM) was prepared in 1.5 mL tube. The primers were used at a concentration of 20 micromole per liter (L). Double distilled water was added to bring the volume of the stock buffer solution to 1.5 mL.For each PCR amplification, 2.0 µL of the target DNA and 2 µL of primers was added to 45 µL of the stock solution in PCR tubes and mixed by vortexing. 1.0 µL of Taq DNA polymerase (Perkin Elmer) was used at a concentration of 5.0 units. All PCR amplification reactions were carried out in a final volume of 50 µL. The thermal cycling profiles were as follows: a 2 min incubation at 94°C, followed by 30 cycles of 94°C for 1 min, 57°C for 30 sec and 72°C for 45 sec. A final incubation at 72°C for 10 min was carried out to ensure complete synthesis of the expected PCR products. PCR reaction products were separated on 1.5% agarose gels, stained with ethidium bromide and visualized. *Listeria monocytogenes* strain (ATCC 7494) and an *E. coli* strain (ATCC 25922) were included as negative and positive controls respectively.

Results

The results of antimicrobial sensitivity test of *E. coli* recovered from human samples indicated that 29/29 strains (100%) were resistant to tetracycline, naldixic acid, ampicillin, trimethoprim, neomycin, oxytetracycline, sulpha-methoxazole trimethoprim, erythromycin and 25/29 strains (86.2%) were sensitive to colistin sulfate (Table 1). On

the other hand, 57/57 (100%) poultry strains were resistant to oxytetracycline and streptomycin, 7/57 (12.3%) were resistant to gentamycin while 100% were sensitive to colistin sulfate.

Table 1. Incidence of antimicrobial sensitivity test on the *E. coli* isolates recovered from human and poultry

Antimicrobial agent	Abbreviation	Resistant				Intermediate				Susceptible			
		Human		Poultry		Human		Poultry		Human		Poultry	
		n=	%	n=	%	n=	%	n=	%	n=	%	n=	%
Naldixic acid (30 µg)	NA	29	100	49	85.9	0	0	0	0	0	0	8	14.1
Tetracycline (30µg)	T	29	100	51	89.5	0	0	0	0	0	0	6	10.5
Doxycycline (30µg)	DO	20	68.9	55	96.5	6	20.7	1	1.8	3	10.3	1	1.8
Gentamycin (10µg)	G	17	58.6	7	12.3	0	0	0	0	12	41.4	50	87.7
Neomycin (10µg)	N	29	100	15	26.4	0	0	21	36.8	0	0	21	36.8
Norfloxacin (5 µg)	NX	28	96.6	36	63.2	0	0	15	26.3	1	3.4	6	10.5
Ciprofloxacin (5µg)	CF	27	93.1	19	33.3	1	3.4	22	38.6	1	3.4	16	28.1
Chloramphenicol (30µg)	C	21	72.4	23	40.4	6	20.7	3	5.3	2	6.9	31	54.4
Colistin sulphate (10 mg)	CL	4	13.8	0	0	0	0	0	0	25	86.2	57	100
Ampicillin (25µg)	A	29	100	48	84.2	0	0	0	0	0	0	9	15.8
Oxytetracycline (30µg)	O	29	100	57	100		0	0	0	0	0	0	0
Streptomycin (10µg)	S	24	82.8	57	100	3	10.3	0	0	2	6.9	0	0
Erythromycin (15µg)	E	29	100	42	73.7	0	0	0	0	0	0	15	26.3
Trimethoprim (5 µg)	TR	29	100	50	87.7	0	0	0	0	0	0	7	12.3
Sulpha-methoxazole trimethoprim 1:19 (25µg)	SXT	29	100	49	85.9	0	0	0	0	0	0	8	14.1

Table 2 records the severe diversity in the antimicrobial resistance profiles of *E. coli* isolates collected from human and poultry (NA, Naldixic acid; T, Tetracycline; DO, Doxycycline; TR, Trimethoprim; G, Gentamycin; N, Neomycin; NX, Norfloxacin; CF, Ciprofloxacin; C, Chloramphenicol; CL, Colistin sulphate; A, Ampicillin; O, Oxytetracycline; S, Streptomycin; E, Erythromycin; SXT, Sulphamethoxazole:trimethoprim).

Table 2. Antimicrobial resistance profiles of *E.coli* isolates collected from poultry and human

Antibiotic resistance profiles	n= of isolates	% of isolates	n= of antibiotics
A-O-S-E	1	1.162	4
NA-T-DO-A-O-S-E	1	1.16	7
NA-T-DO-NX-A-O-S	1	1.16	7
NA-T-DO-NX-A-O-S-E	1	1.16	8
DO-TR-N-NX-O-SXT-S-E	1	1.16	8
DO-TR-G-NX-O-SXT-S-E	1	1.16	8
NA-T-TR-NX-A-O-SXT-S	1	1.16	8
NA-T-DO-TR-A-O-SXT-S	1	1.16	8
NA-T-DO-NX-CF-C-A-O-S	1	2.32	9
NA-T-DO-TR-C-A-O-SXT-S	1	1.16	9
T-DO-TR-G-NX-O-SXT-S-E	1	1.16	9
NA-T-DO-TR-A-O-SXT-S-E	8	10.46	9
T-DO-TR-N-NX-C-O-SXT-S	1	1.16	9
NA-T-DO-TR-C-A-O-SXT-S	1	1.16	9
NA-T-DO-TR-NX-A-O-SXT-S	1	1.16	9
T-DO-TR-N-NX-C-A-O-S-E	1	1.16	10
NA-T-DO-N-NX-CF-C-A-O-S	1	1.16	10
NA-T-DO-TR-C-A-O-SXT-S-E	1	1.16	10
NA-T-TR-G-N-NX-A-O-SXT-E	1	1.16	10
NA-DO-TR-G-N-X-O-SXT-S-E	2	2.32	10
NA-T-TR-N-NX-A-O-SXT-S-E	1	1.16	10
NA-T-DO-TR-C-A-O-SXT-S-E-	1	1.16	10
NA-T-DO-TR-X-A-O-SXT-S-E	3	3.5	10
NA-T-DO-TR-CF-A-O-SXT-S-E	2	2.32	10
NA-T-DO-TR-CF-C-A-O-SXT-S	1	1.16	10
NA-T-DO-TR-NX-A-O-SXT-S-E	1	1.16	10
NA-T-TR-N-NX-CF-A-O-SXT-E	1	1.16	10
NA-T-DO-TR-NX-C-A-O-SXT-S	1	1.16	10
NA-T-TR-N-NX-CF-A-O-SXT-E	1	1.16	10
NA-T-DO-TR-CF-C-A-O-SXT-S	1	1.16	10
NA-T-DO-TR-N-NX-C-A-O-SXT	1	1.16	10
NA-T-DO-TR-G-N-NX-C-O-SXT-S	1	1.16	11
NA-T-DO-TR-N-CF-C-A-O-SXT-E	1	1.16	11
NA-T-DO-G-N-NX-A-O-SXT-S-E	1	1.16	11
NA-T-TR-N-NX-CF-A-O-SXT-S-E	2	2.32	11
NA-T-DO-TR-CF-C-A-O-SXT-S-E	1	1.16	11
T-DO-TR-N-NX-CF-C-O-SXT-S-E	1	1.16	11
NA-T-DO-TR-NX-C-A-O-SXT-S-E	1	1.16	11
NA-T-TR-N-NX-CF-C-A-O-SXT-E	1	1.16	11
NA-T-DO-TR-N-NX-A-O-SXT-S-E	1	1.16	11
NA-T-DO-TR-NX-CF-A-O-SXT-S-E	4	4.65	11
NA-T-DO-TR-N-NX-CF-C-A-O-S-E	1	1.16	12
NA-T-DO-TR-G-N-NX-A-O-SXT-S-E	1	1.16	12
NA-T-DO-TR-N-NX-CF-C-O-SXT-S-E	1	1.16	12
NA-T-DO-TR-N-NX-CF-A-O-SXT-S-E	2	2.32	12
NA-T-DO-TR-NX-CF-C-A-O-SXT-S-E	4	4.65	12
NA-T-TR-G-N-NX-CF-C-A-O-SXT-S-E	2	2.32	13
NA-T-DO-TR-G-N-NX-CF-A-O-SXT-S-E	1	1.16	13
NA-T-DO-TR-N-NX-CF-C-A-O-SXT-S-E	5	5.81	13
NA-T-DO-TR-G-N-NX-CF-C-A-O-SXT-E	1	1.16	13
NA-T-TR-N-NX-CF-A-C-O-SXT-E-S-G-CL	1	1.16	14
NA-T-DO-TR-G-N-NX-CF-C-A-O-SXT-S-E	8	10.46	14
NA-T-DO-TR-G-N-NX-CF-C-CL-A-O-SXT-S	1	1.16	14
NA-T-DO-TR-G-N-NX-CF-C-CL-A-O-SXT-S-E	2	2.32	15

Figure 1.Tahrir Square in Cairo on July 26, 2013

Discussion

In Egypt, the antibiotics are consumed with high rate of consumption and their consumption is associated with a high rate of misuse. Misuse is caused by unwarranted prescription, inappropriate choice of treatment, self-prescription, lack of adherence by consumers, as well as lax regulation on the use of antibiotics. This misuse and overuse of antibiotics is particularly important because it contributes to selection and increased occurrence of antimicrobial resistant bacteria.

The growing problem of multidrug-resistant enteric pathogens is especially common in Latin America, Africa and Asia (Nys et al., 2004; Bii et al., 2005; Okeke et al., 2005; Hien et al., 2008). Ochoa et al. (2009) found a high frequency of antimicrobial resistance of diarrheagenic *E. coli* to commonly used antibiotics such as ampicillin, tetracyclines, aminoglycosides and sulfonamides. This finding is similar to what has been recently described in children from Argentina (Binsztein et al., 1999), Tanzania (Vila et al., 1999), México (Estrada-García et al., 2005), Vietnam (Nguyen et al., 2005), Indonesia (Duerink et al., 2007), Mozambique (Mandomando et al., 2007) and Colombia (Go´mez-Duarte et al., 2010). Similar to the findings of previous studies (Bass et al., 1999; Yang et al., 2004), antimicrobial resistance in *E. coli* isolates may vary according to the animal host it was isolated from. Chicken *E. coli* isolates may exhibit a higher degree of resistance than beef isolates (de Jong et al., 2009; Ewers et al., 2009). Research has also showed that there is a potential human origin to antibiotic resistance in bacteria from domestic/companion animals (Johnson et al., 2008; Pomba et al., 2009) as *E. coli* isolated from domestic/companion animals tend to present the same resistance and virulence of *E. coli* isolated from humans.

Many drug-resistant human fecal *E. coli* isolates may originate from poultry, whereas drug-resistant poultry-source *E. coli* isolates likely originate from susceptible poultry-source precursors. A third generation of cephalosporin, e.g. cefotaxime and ciprofloxacin, are critically important antimicrobials for human medicine. APEC strains can present high levels of resistance to tetracycline, sulfamethoxazole and fluoroquinolones (Wang et al., 2010). Acquired resistance to first-line antimicrobial agents increasingly complicates the management of extraintestinal infections due to *E. coli*, which are a major source of illness, death, and increased healthcare costs (Garau et al., 1999; Gupta et al., 2001; Russo and Johnson, 2003; Pitout et al., 2005). One suspected source of drug-resistant *E. coli* in humans is use of antimicrobial drugs in agriculture. This use presumably selects for drug-resistant *E. coli*, which may be transmitted to humans through the food supply (Linton, 1977; Jones et al., 2005; Collignon and Angulo, 2006). Supporting this hypothesis is the high prevalence of antimicrobial drug–resistant *E. coli* in retail meat products, especially poultry (Johnson et al., 2003, 2005a,b, 2006; Schroeder et al., 2003), and the similar molecular characteristics of fluoroquinolone-resistant *E. coli* from chicken carcasses and from colonized and

infected persons in Barcelona, Spain, in contrast to the marked differences between drug-susceptible and drug-resistant source isolates from humans (Mellon et al., 2001).

In the present investigation, we analyzed the drug-susceptible and drug resistant *E. coli* isolates from human isolates and poultry flocks in Egypt. We found that drug resistant human isolates, although overlapping somewhat with drug-susceptible human isolates, were more similar overall to poultry isolates than to drug-susceptible human isolates. In contrast, drug-susceptible human isolates differed from poultry isolates. This relationship was observed consistently with diverse analytical approaches and various stratifications of the population. It suggests that many of the drug-resistant human isolates were more likely to have originated in poultry and to have been acquired by humans when these isolates were already drug resistant, than to have emerged de novo in humans by conversion of drug-susceptible human isolates to drug-resistant isolates. We also found that, regardless of analytical approach and population analyzed, resistant and susceptible poultry isolates were highly similar. This suggests that the resistant poultry isolates likely derived from antimicrobial drug–susceptible, poultry-source *E. coli* by conversion to resistance. This most plausibly would occur within the avian fecal flora under selection pressure from on-farm use of antimicrobial drugs. This implies that drug-resistant poultry-source *E. coli* isolates originate in the birds, rather than being introduced from some exogenous reservoir later during the packaging and distribution process. This in turn suggests that on-farm practices, including use of antimicrobial agents for growth promotion, metaphylaxis, and therapy, may influence characteristics of *E. coli* that contaminate retail poultry products and, seemingly, are then transmitted to humans.

The role of poultry as a source of human ExPEC is suggested by multiple epidemiological studies that reveal the presence of avian ExPEC in both the intestines of healthy poultry and poultry meat from retail markets, strains that are often genetically similar to those found to be responsible for human infections (Manges and Johnson, 2012). ExPEC transmission from food animals could be responsible for human infections, and chickens are the most probable reservoir (Vincent et al., 2010; Bergerone et al., 2012). Many lines of evidence link antimicrobial-resistant human infections to foodborne pathogens of animal origin (Swartz, 2002; Vincent et al., 2010). The appearance and increase of antibiotic resistance among ExPEC strains complicate the therapeutic management of ExPEC infections (Smith et al., 2007) and has led to the increased use of last-resort antimicrobial drugs, such as carbapenems, and the appearance of the resistance to these antibiotics in ExPEC. Broilers were found to be a source for ciprofloxacin-resistant human-derived *E. coli* (Thorunn et al., 2010). Vincent et al. (2010) described the results of a study that characterized the genetic similarities between *E. coli* isolates recovered from retail meat, particularly chicken, and ExPEC in humans causing community-acquired UTIs (Vincent et al., 2010). That study suggested that chicken was likely to be the primary reservoir of ExPEC in humans (Schroeder et al., 2003; Johnson et al., 2005a,b; 2006; 2007; Cortés et al., 2010; Bergeron et al., 2012).

The 86 *E. coli* isolates characterized in this study displayed resistance to one or more antimicrobials, including penicillins, sulfonamides, chloramphenicol, trimethoprim-sulfamethoxazole, or amoxicillin-clavulanic acid; cephalosporins, tetracyclines, and aminoglycosides. These data are in accord with multiple previous studies suggesting use of these drugs has been a key factor in the emergence of antimicrobial-resistant *E. coli* (Meng et al., 1998; Bass et al., 1999; Teshager et al., 2000; Threlfall et al., 2000; Stephan et al., 2001; Zhao et al., 2001; Schroeder et al., 2002a,b; Yang et al., 2004).

Clinicians are aware that the increase in antibiotic resistance is a great concern for public health as well as the economy. Treatment failures due to ATB resistance increase the cost of care and result in prolonged morbidity for patients. As the proportion of elderly and immunocompromised patients increases, the number of ExPEC infections will likely increase, while associated antibiotic resistance will make treatment strategies more challenging (Pitout, 2012). Therefore, the prevention of ExPEC infections is a pressing concern.

Because the trimethoprim-sulfamethoxazole combination is recommended for treating a range of human infections, including complicated urinary tract infections, acute uncomplicated cystitis, and pyelonephritis (Thielman and Guerrant, 1999), *E. coli* isolates should be monitored for further dissemination of trimethoprim-sulfamethoxazole resistance. Virtually all trimethoprim-sulfamethoxazole-resistant isolates from this study, however, were susceptible to ciprofloxacin and ceftriaxone, both of which are important antimicrobials for treating infections caused by trimethoprim-sulfamethoxazole-resistant *E. coli*. Diarrheagenic *E. coli* as a group were found to exhibit high levels of antimicrobial drug resistance in diarrheal cases to ampicillin, cotrimoxazole, tetracycline and nalidixic acid (Ochoa et al., 2009).

The multiple antimicrobial-resistant phenotypes observed in this study may have resulted from the spread of mobile genetic elements. For example, the observation that nearly 75% of ampicillin-resistant *E. coli* isolates were also resistant to streptomycin and tetracycline suggests resistance genes for these drugs are linked on plasmids. Moreover, the widespread resistance to sulfamethoxazole implies the presence of class I integrons, which are also important in conferring resistance to multiple antimicrobials (Jones et al., 1997).

Data from this study suggest that antimicrobial use in clinical medicine and in agriculture was important in the selection of antimicrobial-resistant phenotypes. Continued surveillance of *E. coli* collected from agricultural and clinical settings, including the food production continuum, is merited to identify emerging antimicrobial-resistant phenotypes.

Also, these results can be used to identify important environmental risk factors for the diseases and to identify the communities where background risk is highest. Limited public health resources can then be targeted to the risk factors and communities most at risk. These results can also be used as the framework upon which to develop a comprehensive epidemiological study that focuses on risk factors important at the individual level.

Our assessment to the risks of antimicrobial-resistant phenotypes that are associated with MGs, outline approaches to risk assessment and mitigation, and draw attention to some key challenges encountered by organisers and participants. Crowding and lack of sanitation at MGs can lead not to the emergence of infectious diseases only, but also to the rapid dissemination of antimicrobial-resistant phenotypes through population movement which can spread them nationally and internationally posing huge challenges to planners of MGs and clinicians; however, these events also provide an opportunity to engage in public health action that will benefit host communities and the regions from which participants originate. Therefore, antimicrobial-resistant surveillance for MGs can be directed locally and globally.

In conclusion, to prevent the spread of antibiotic resistance the impact of MGs on the spread of MDR-*E. coli* has become more evident.

References

Bass, L., Liebert, CA, Lee, M.D., Summers, A.O., White, D.G., Thayer, S.G. and Maurer, J.J. (1999): Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. Antimicrob.Agents Chemother. 43:2925–2929.

Bergeron, C.R., Prussing, C., Boerlin, P., Daignault, D., Dutil, L., Reid-Smith, R.J., Zhanel, G.G. and Manges, A.R. (2012): Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. Emerg. Infect. Dis. 18:415–421.

Bii, C.C., Taguchi, H., Ouko, T.T., Muita, L.W., Wamae, N. and Kamiya, S. (2005): Detection of virulence-related genes by multiplex PCR in multidrug-resistant diarrhoeagenic *Escherichia coli* isolates from Kenya and Japan. Epidemiol. Infect. 133:627–633.

Binsztein, N., Picandet, A.M., Notario, R., Patrito, E., De Lesa, M.E., De Petris, A., Maurel, D., Nader, O., Rivas, M., Szefner, M. and Vergara, M. (1999): Antimicrobial resistance among species of *Salmonella*, *Shigella*, *Escherichia*, and *Aeromonas* isolated from children with diarrhea in 7 Argentinian centers. Rev. Latinoam. Microbiol. 41:121–126.

CLSI (2010): Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility

Collignon, P. and Angulo, F.J. (2006): Fluoroquinolone-resistant *Escherichia coli*: food for thought. J. Infect. Dis. 194:8–10.

Cortés, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J.E., Blanco, M., Lo´pez, C.,Andreu, A., Navarro, F., Alonso, M.P., Bou, G., Blanco, J. and Llagostera, M. (2010): Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl. Environ. Microbiol. 76:2799–805

de Jong, A., Bywater, R., Butty, P., Deroover, E., Godinho, K., Klein, U., Marion, H., Simjee, S., Smets, K., Thomas, V., Vallé, M. and Wheadon, A. (2009): A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. J. Antimicrob. Chemother. 63:733-44

Doyle, M.P., Loneragan, G.H., Scott, H.M. and Singer, R.S. (2013): Antimicrobial Resistance: Challenges and Perspectives. Comp. Rev. Food Sci. Food Safety 12:234–248.

Duerink, D.O., Lestari, E.S., Hadi, U., Nagelkerke, N.D., Severin, J.A., Verbrugh, H.A., Keuter, M., Gyssens, I.C. and van den Broek, P.J. (2007): Determinants of carriage of resistant *Escherichia coli* in the Indonesian population inside and outside hospitals. J. Antimicrob. Chemother. 60: 377–384.

Estrada-García, T., Cerna, J.F., Pacheco-Gil, L., Velázquez, R.F., Ochoa, T.J., Torres, J. and DuPont, H.L. (2005): Drug-resistant diarrheogenic *Escherichia coli*, Mexico. Emerg. Infect. Dis. 11:1306–1308.

Ewers, C., Antao, E.M., Diehl, I., Philipp, H.C. and Wieler, L.H. (2009): Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. Appl. Environ.Microbiol. 75:184–192.

Garau, J., Xercavins, M., Rodriguez-Carballeira, M., Gomez-Vera, J.R., Coll, I. and Vidal, D. (1999): Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob.Agents Chemother. 43:2736–2741.

Go´mez-Duarte, O.G., Arzuza, O., Urbina, D., Bai, J., Guerra, J., Montes, O., Puello, M., Mendoza, K. and Castro, G.Y. (2010): Detection of *Escherichia coli* Enteropathogens by Multiplex Polymerase Chain Reaction from Children's Diarrheal Stools in Two Caribbean–Colombian Cities. Foodborne Pathog. Dis. 7:199-206.

Gupta, K., Hooton, T.M. and Stamm, W.E. (2001): Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann. Intern. Med. 135:41–50.

Heininger, A., Binder, M., Schmidt, S., Unertl, K., Botzenhart, K. and Döring, G. (1999): PCR and blood culture for detection of *Escherichia coli* bacteremia in rats. J. Clin. Microbiol. 37:2479–2482.

Hien, B.T., Scheutz, F., Cam, P.D., Serichantalergs, O., Huong, T.T., Thu, T.M. and Dalsgaard, A. (2008): Diarrheagenic *Escherichia coli* and *Shigella* strains isolated from children in a hospital case-control study in Hanoi, Vietnam. J. Clin. Microbiol. 46:996–1004.

Johnson, J.R., Clabots, C. and Kuskowski, M.A.(2008): Multiple-host sharing, long-term persistence, and virulence of *Escherichia coli* clones from human and animal household members. J. Clin. Microbiol. 46:4078-4082.

Johnson, J.R., Delavari, P., O'Bryan, T.T., Smith, K. and Tatini, S. (2005a): Contamination of retail foods, particularly turkey, from community markets (Minnesota, 1999–2000) with antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog. Dis. 2:38–49.

Johnson, J.R., Kuskowski, M.A., Menard, M., Gajewski, A., Xercavins, M. and Garau, J. (2006): Similarity of human and chicken-source *Escherichia coli* isolates in relation to ciprofloxacin resistance status. J. Infect. Dis. 194:71–78.

Johnson, J.R., Kuskowski, M.A., Smith, K., O'Bryan, T.T. and Tatini, S. (2005b): Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. J. Infect. Dis. 191:1040–1049.

Johnson, J.R., Murray, A.C., Gajewski, A., Sullivan, M., Snippes, P. and Kuskowski, M.A. (2003): Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob. Agents Chemother. 47:2161–2168.

Johnson, J.R. and Russo, T.A. (2002): Extraintestinal pathogenic *Escherichia coli*: "the other bad *E coli*". J. Lab. Clin. Med. 139:155-162.

Johnson, T.J., Kariyawasam, S., Wannemuehler, Y., Mangiamele, P., Johnson, S.J., Doetkott, C., Skyberg, J.A., Lynne, A.M., Johnson, J.R. and Nolan, L.K. (2007): The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. J. Bacteriol. 189:3228–3236.

Jones,M.E., Peters,E., Weersink,A.M., Fluit,A. and Verhoef, J. (1997): Widespread occurrence of integrons causing multiple antibiotic resistance in bacteria. Lancet 349:1742–1743.

Jones, T.F. and Schaffner, W. (2005): New perspectives on the persistent scourge of foodborne disease. J. Infect. Dis. 191:1029–1031.

Linton, A.H. (1977): Animal to man transmission of Enterobacteriaceae. R. Soc. Health. J. 97:115–118.

Mandomando, I.M., Macete, E.V., Ruiz, J., Sanz, S., Abacassamo, F., Vallès, X., Sacarlal, J., Navia, M.M., Vila, J., Alonso, P.L. and Gascon, J. (2007): Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of southern Mozambique. Am. J. Trop. Med.Hyg. 76:522-527.

Manges, A.R. and Johnson, J.R. (2012): Food-borne origins of *Escherichia coli* causing extraintestinal infections. Clin. Infect. Dis. 55:712–719.

Mellon, M., Benbrook, C. and Lutz Benbrook, K. (2001): Hogging it. Estimates of antimicrobial abuse in livestock. Cambridge (MA): UCS Publications.

Meng, J., Zhao, S., Doyle, M.P. and Joseph, S.W. (1998): Antibiotic resistance of *Escherichia coli* O157:H7 and O157:NM isolated from animals, food, and humans. J. Food Prot. 61:1511–1514.

Nguyen, T.V., Le Van, P., Le Huy, C.,Gia, K.N. and Weintraub, A. (2005): Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. J. Clin. Microbiol. 43:755–760.

Nys, S., Okeke, I.N., Kariuki, S., Dinant, G.J., Driessen, C., and Stobberingh, E.E. (2004): Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries. J. Antimicrob. Chemother. 54:952–955.

Ochoa, T.J., Ruiz, J., Molina, M., Del Valle, L.J., Vargas, M., Gil, A.I., Ecker, L., Barletta, F., Hall, E., Cleary, T.G. and Lanata, C.F. (2009): High Frequency of Antimicrobial Drug Resistance of Diarrheagenic *Escherichia coli* in Infants in Peru. Am. J. Trop. Med. Hyg. 81:296–301.

Okeke, I.N., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A. and Klugman, K.P. (2005): Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect. Dis. 5:481–493.

Pitout, J.D. (2012): Extraintestinal pathogenic *Escherichia coli*: A combination of virulence with antibiotic resistance. Front. Microbiol. 3:1–7.

Pitout. J.D., Nordmann, P., Laupland, K.B. and Poirel, L. (2005): Emergence of Enterobacteriaciae producing extended spectrum β-lactamases (ESBLs) in the community. J. Antimicrob. Chemother. 56:52–59.

Pomba, C., da Fonseca, J.D., Baptista, B.C., Correia, J.D. and Martínez-Martínez, L. (2009): Detection of the pandemic O25-ST131 human virulent *Escherichia coli* CTXM-15-producing clone harboring the qnrB2 and aac (6')-Ib-cr genes in a dog. Antimicrob. Agents Chemother. 53:327-328.

Russo, T.A. and Johnson, J.R. (2003): Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes Infect.5:449-456.

Schroeder, C.M., White, D.G., Ge, B., Zhang, Y., McDermott, P.F. and Avers, S. (2003): Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. Int. J. Food Microbiol. 85:197–202.

Schroeder, C.M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D.G., Wagner, D.D., McDermott, P.F., Walker, R.D. and Meng, J. (2002a): Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. Appl. Environ. Microbiol. 68:576–581.

Schroeder, C.M., Meng, J., Zhao, S., DebRoy, C., Torcolini, J., Zhao, C., McDermott, P.F., Wagner, D.D., Walker, R.D. and White, D.G. (2002b): Antimicrobial Resistance of *Escherichia coli* O26, O103, O111, O128, and O145 from Animals and Humans. Emerg. Infect. Dis. 8:1409-1414.

Smith, J.L., Fratamico, P.M. and Gunther, N.W. (2007): Extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog. Dis. 4:134-163.

Stephan, R. and Schumacher, S. (2001): Resistance patterns of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from animals, food and asymptomatic human carriers in Switzerland. Lett. Appl. Microbiol. 32:114–117.

Swartz, M.N. (2002): Human Disease and Foodborne Pathogens. CID 34 (Suppl 3): S111-S122.

Teshager, T., Herrero, I.A., Porrero, M.C., Garde, J., Moreno, M.A. and Dominguez, L. (2000): Surveillance of antimicrobial resistance in *Escherichia coli* strains isolated from pigs at Spanish slaughterhouses. Int. J. Antimicrob. Agents 15:137–142.

Thielman,N.M. and Guerrant,R.L. (1999): *Escherichia coli*. In: Yu, V.L., Merigan,Jr T.C. and Barriere, S.L., editors. Baltimore: The Williams & Wilkins Company: p. 188–200.

Thorsteinsdottir, T.R., Haraldsson, G., Fridriksdottir, V., Kristinsson, K.G. and Gunnarsson, E. (2010): Broiler Chickens as Source of Human Fluoroquinolone-Resistant *Escherichia coli*, Iceland. Emerg. Infect. Dis. 16:133-135.

Threlfall, E.J., Ward, L.R., Frost, J.A. and Willshaw, G.A. (2000): The emergence and spread of antibiotic resistance in food-borne bacteria. Int. J. Food Microbiol. 62:1–5.

Toole, M.J. (1997): Communicable diseases and disease control: In: Noji, E. ed. Public health consequences of disasters. New York, Oxford University Press.

Vila, J., Vargas, M., Casals, C., Urassa, H., Mshinda, H., Schellemberg, D. and Gascon, J. (1999): Antimicrobial resistance of diarrheagenic *Escherichia coli* isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrob.Agents Chemother. 43:3022–3024.

Vincent, C., Boerlin, P., Daignault, D., Dozois, C.M., Dutil, L., Galanakis, C., Reid-Smith, R.J., Tellier, P.P., Tellis, P.A., Ziebell, K. and Manges, A.R. (2010): Food Reservoir for *Escherichia coli* Causing Urinary Tract Infections. Emer. Infect. Dis.16:88-95.

Woodford, N, Wareham, D.W., Guerra, B.and Teale, C. (2014): Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? J. Antimicrob. Chemother. 69:287-291.

Yang, H., Chen, S., White, D.G., Zhao, S., McDermott, P., Walker, R. and Meng, J. (2004): Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J. Clin. Microbiol. 42:3483–3489.

Zhao, S., White, D.G., Ge, B., Ayers, S., Friedman, S. and English, L. (2001): Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin–producing *Escherichia coli* isolates. Appl. Environ.Microbiol. 67:1558–1564.