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Water Quality Assessment of Swimming Pools and Risk of Spreading Infections in Ghana

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ABSTRACT

The quality of drinking water in most African countries is a call for concern let alone the quality of swimming pools. A very few information exist about the quality of swimming pools in Africa because most researchers concentrate on the quality of drinking water. However, the quality of swimming pool water must be as good as drinking water due to the risk of exposure to the body orifices during swimming. Seven different swimming pools in Ghana were investigated for their water qualities and the risk of spreading pathogenic and antibiotic resistant microorganisms to swimmers. Four samples each were collected purposively from each swimming pool for analysis over four months (December 2013-March 2014) period. Standard microbiological procedures were followed to isolate fecal coliforms, *E. coli*, total heterotrophic bacteria as well as antibiotic susceptibility test of isolated *E. coli* to commonly used antibiotics using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Physicochemical parameters such pH, free chlorine and turbidity were also measured. Bacteria isolated include fecal coliforms (13-36 CFU/100 mL), *E. coli* (5-8 CFU/100 mL) and Heterotrophic bacteria (26-90 CFU/1 mL) from all swimming pools and *E. coli* O157:H7 from two pools. The antibiotic that recorded the highest resistance was sulfamethoxazole (46%) followed by amoxicillin (29%), ceftriaxone (25%), chloramphenicol (21%), amoxicillin clavulanic acid (14%), ciprofloxacin (11%) and gentamicin (4%). The pH values ranged from 6.2-7.2. The free chlorine concentration obtained ranged from 1.3-1.9 mg L⁻¹ and Nephelometric Turbidity Unit ranged from 1.1-1.7 NTU. The presence of high levels of fecal coliform bacteria and *E. coli* in the seven swimming pools have not met the World Health Organization (WHO) standard for recreational waters. Also, the swimming pools were below standards with regards to pH and turbidity. The antibiotic resistant isolates found in the swimming pools can easily spread to swimmers. Swimmers in pools in Ghana are at a risk of contracting infectious diseases, hence urgent and effective mitigating interventions must be devised to ensure standard measures.

Key words: Swimming pools, water quality, indicator organisms, *E. coli* O157:H7, antibiotic resistance, Ghana, Africa

INTRODUCTION

Recreation is a very important component in the life of people in the world and it comes in different forms. The availability of recreational facilities determines the type of recreational

activities people patronize. One of the cheapest ways of recreating oneself is by the use of nature's own available water resources like rivers, dams, lakes, seas etc. To make recreation with water more accessible and convenient, people construct swimming pools in houses, hostels, hotels, schools and other public places. With evolving and advancing civilization, man-made water recreational environments are on the boom but also presenting certain extent of risk of microbiological and chemical contaminations (Barna and Kadar, 2012). Several pathogens can be transmitted into the human body due to swimming and infected water through the body orifices.

However, very little information exists when it comes to the quality of water for recreation and especially swimming pools in Africa as well as West African (Abdou *et al.*, 2005; Van Heerden *et al.*, 2005; Abd El-Salam, 2012). There is little information about pathogenic *E. coli* in Africa (Muller *et al.*, 2001). There is currently little or no data about infections and outbreak of diseases in swimming pools in Ghana although there is a high risk of infections due to non-compliance and non-enforcement of regulations regarding the establishment and maintenance of swimming pools. Those who normally take care of these swimming pools have little knowledge about the importance of maintaining the pools to meet both the microbiological and physiochemical standards. Some tend to economize chemicals use for sanitizing the pools due to their scarcity or over chlorinate the pools due to little knowledge of the recommended quantities to apply and hence compromise the quality of the swimming pools. Under application of chlorine will help microorganisms to thrive in the water and over application may lead to toxic effects on swimmers (Martinez and Long, 1995; Bernard *et al.*, 2003). These expose swimmers to myriads of infections knowing that the average amount of water swallowed by non-adults and adults during a normal swimming session is approximately 37 and 16 mL, respectively (Dufour *et al.*, 2006). These volumes may be more in those who do not know or are learning how to swim. This is likely to be the case of most swimmers in Ghana and Africa, where swimming courses are not part of the normal training during infancy. Pathogenic *E. coli* O157:H7 has been implicated in a number of outbreaks in recreational waters in the developed countries in Scotland (Brewster *et al.*, 1994), Portland, Oregon, USA (Keene *et al.*, 1994) and South West London (Hildebrand *et al.*, 1996) but little information exist here in Ghana and Africa about their presence in swimming pools. There is, therefore, the need to investigate the health and hygiene of swimming pools for microbial (indicator organisms) and physiochemical parameters in Ghana to ascertain the extent of the contamination levels in swimming pools as well as compliance of swimming pools to international standards.

In this study, we determined the microbial and physiochemical water quality of seven public and private (hotels) swimming pools in a district that is known for its tourist potentials in the Eastern Region of Ghana and compared the parameters to the World Health Organization standards for recreational waters (WHO., 2006).

MATERIALS AND METHODS

Sample collection, handling and transportation: A total of 28 water samples were purposively collected four times from seven different public and private (hotels) with swimming pools in a district over a four-month period (December 2013 to March 2014). Samples were collected and transported to the laboratory in a flask containing ice cubes for analysis within 15 min. The sampling sites were located at site A, B, C, D, E, F and G. The site D is a public pool and open to the general public with a token while site E is semi-public. Sites A, B, C, F and G are swimming pools attached to hotels and resort and are mostly used by costumers who patronize the hotels and resorts. The bather loads per day of the swimming pools ranges from 18-40 people.

Physiochemical parameters determination: Temperature, pH, electrical conductivity and turbidity of the swimming pools water were determined *in situ* using the Pelintest multi-purpose meter. Free chlorine was determined with the aid of a 10 mL calibrated test tube. Ten milliliter of each sample was pipetted into the test tube and a reagent tablet Diethyl-p-Phenylene Diamine (DPD), was crushed in the sample and allowed for 5 min and inserted into the direct reading spectrophotometer and set to 530 nm wave length to record the level of free chlorine in the various samples just before swimming.

Microbiological analysis of swimming pool water: Membrane filtration technique was employed to determine fecal coliforms and *Escherichia coli* in accordance with American Public Health Association (APHA), 9222D and 9260F. Filtration unit comprising of Erlenmeyer flask, vacuum source and porous support were assembled and with the aid of a flame-sterilized forceps, a sterile membrane filter (0.45 µm Millipore) was placed on the porous support. The upper funnel was placed in position and secured with appropriate clamps in a Millipore machine. The 100 mL of the swimming pool water sample was aseptically poured into the upper funnel and suction applied to create a vacuum. After the sample passed through the membrane filter, the filtration unit was taken apart and with the aid of a sterilized forceps the membrane filter was placed in the Petri dish containing selective media M-FC agar (Membrane filter-Fecal Coliform agar) for the determination of fecal coliform and Hi-Chrome *E. coli* agar for *E. coli*. Clamps, forceps and new Petri dish were usually sterile prior to use for the next sample. All plates were labelled and incubated in inverted position at 37±2°C for *Escherichia coli* and 44±2°C for fecal coliform for 18-24 h. After incubation the light grey-blue colour of the M-FC agar for fecal coliform changes to greyish-beige colour indicating the colonies of the fecal coliform and a light bluish gel of the Hi-Chrome *E. coli* agar turns bluish-green indicating the presence of *E. coli* colonies.

Determination of Total Heterotrophic Bacteria (THB): Total heterotrophic bacteria count was determined following the heterotrophic plate count method, using pour plate technique in accordance with APHA 9215. One milliliter of the sample was pipette into a sterilized Petri dish and 10-15 mL of nutrient agar (Oxoid, Basingstoke, Hampshire, England) added to it. This was uniformly mixed for a minute and allowed to solidify. It was then incubated at 37°C for 48 h. Colony growth on the plate after the incubation period was then counted using colony counter.

Detection of Shiga-toxin producing *E. coli* (STEC) on CHROMagar STEC media: Four *E. coli* isolates from the Hi-chrome agar were randomly picked and streaked on MacConkey agar (Oxoid, Basingstoke, Hampshire, England) to further confirm their lactose fermenting ability. The four *E. coli* isolates were also streaked on CHROMagar STEC (www.CHROMagar.com), a specific chromogenic agar for the isolation of Shiga-toxin producing *E. coli* isolates that produced Shiga-toxins. Shiga-toxin isolate on the CHROMagar STEC agar form mauve colonies.

Detection of *E. coli* O157:H7 from non-*E. coli* O157:H7 serotypes: Sorbitol MacConkey agar (Oxoid, Basingstoke, Hampshire, England) was used to differentiate *E. coli* O157:H7 from non-*E. coli* O157:H7 serotypes. The STEC isolates that were picked from the CHROMagar STEC agar were streaked on Sorbitol MacConkey agar to test their ability to utilize sorbitol. The non-*E. coli* O157:H7 serotypes were able to utilize sorbitol with a pink/red colonies whiles the *E. coli* O157:H7 could not utilize sorbitol but produced colorless colonies.

Antibiotics susceptibility test: Antibiotic susceptibility test was performed for all the isolates using the Kirby Bauer disk (Bauer *et al.*, 1966) diffusion method with seven different antibiotic disks (bioMerieux, España, S.A) namely: Amoxicillin (25 µg), amoxicillin clavulanate (20/10 µg), sulfamethoxazole (25 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg) and ceftriaxone (30 µg). The susceptibility and resistance of the isolates were determined by using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for 2014. Multiple drug resistant isolates were designated as resistance to 3 or more antibiotics.

Statistical analysis: Microsoft excel software was used in drawing graphs and tables.

RESULTS

Mean microbial and physiochemical parameters: Table 1 showed the mean microbial and physicochemical parameters measured during this study. Most of the swimming pools sampled (86%) fall below the recommended pH range (7.2-7.8) by WHO. Temperature, electrical conductivity, free chlorine concentration and total heterotrophic bacteria were all within the recommended limits of WHO except for the turbidity (1.1-1.7 NTU) that recorded higher values far beyond the recommended value of 0.5 NTU. Fecal coliform was recorded in all the swimming pools sampled ranging from 13-36 CFU 100 mL⁻¹. *Escherichia* was also present in all the swimming pool water sampled ranging from 5-8 CFU 100 mL⁻¹. Both fecal coliform and *E. coli* were above the recommended limit of zero by WHO. The total heterotrophic bacteria ranged from 26-90 CFU/1 mL and falls below the recommended value of 200 CFU/1 mL by WHO (2006). The highest colonies of fecal coliform, *E. coli* and total heterotrophic bacteria were recorded at site D which is a public swimming pool.

***Escherichia* O157:H7 from non-*E. coli* O157:H7 serotypes:** Two of the 28 *E. coli* tested for the production of Shiga toxin on CHROMagar STEC agar were positive. The positive STEC samples were from site D (public) and E (semi-public). The results on the sorbitol MacConkey agar showed that the two isolates were *E. coli* O157:H7 strains due to their inability to ferment sorbitol.

Antibiotic susceptibility testing: All twenty-eight isolated *E. coli* samples from the confirmatory analysis and two others from the *E. coli* O157:H7 serotype test were subjected to seven different common antibiotics namely: Ceftriaxone, chloramphenicol, gentamicin, ciprofloxacin, amoxicillin clavulanic acid, sulfamethoxazole and amoxicillin. The antibiotic susceptibility results of the isolates

Table 1: Mean values of the parameters measured from the various swimming pools

Parameters	A	B	C	D	E	F	G	WHO limits
pH	7.2	6.8*	6.6*	7.0*	7.1*	6.2*	6.8*	7.2-7.8
Temperature (°C)	22	27	26	26	25	25	24	21-32
EC (µS cm ⁻¹)	77	87	76	87	76	72	83	150
Turbidity (NTU)	1.2*	1.1*	1.1*	1.5*	1.6*	1.6*	1.7*	0.5
Chlorine (mg L ⁻¹)	1.9	1.8	1.5	1.3	1.6	1.6	1.6	<3
THB (CFU/1 mL)	26	28	46	90	62	56	69	<500
FC (CFU/100 mL)	13*	24*	27*	36*	30*	29*	23*	<1
<i>E. coli</i> (CFU/100 mL)	5*	8*	8*	10*	5*	9*	8*	<1

*Compared to the WHO limits, EC: Electrical conductivity, THB: Total heterotrophic bacteria, FC: Fecal coliform

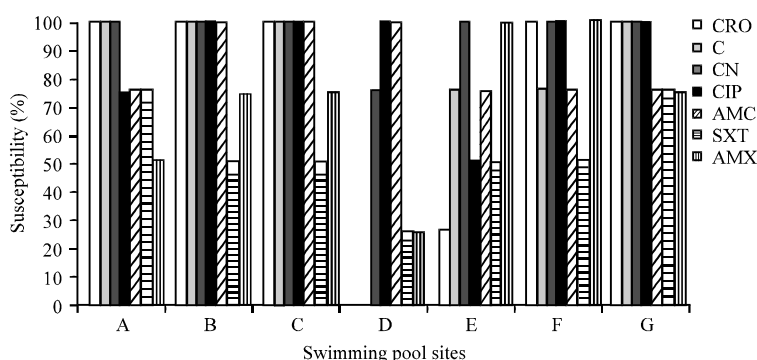


Fig. 1: Percentage of susceptibility levels of isolated *E. coli* to antibiotics used for the various swimming pools sampled, Site A, B, C, F and G are swimming pools attached to hotels and resorts, Site D is a public pool and Site E is semi-public, CRO: Ceftriaxone, C: Chloramphenicol, CN: Gentamicin, CIP: Ciprofloxacin, AMC: Amoxicillin clavulanic acid, SXT: Sulfamethoxazole and AMX: Amoxicillin

exhibited various susceptibility levels towards the seven antibiotics used as shown in Fig. 1. The sampling site, where *E. coli* isolates recorded the highest resistance level to all the antibiotics used was site D (public swimming pool) followed by site E (semi-public), F, G, A, B and C in the order of decreasing resistance which are all swimming pools attached to hotels or resorts. The antibiotic that recorded the highest resistance was sulfamethoxazole (46%) followed by amoxicillin (29%), ceftriaxone (25%), chloramphenicol (21%), amoxicillin clavulanic acid (14%), ciprofloxacin (11%) and gentamicin (4%). From the supplementary Table 1, among all the 28 *E. coli* isolates, 7 (25%) isolates were susceptible to all the seven antibiotics, 11 (39%) isolates were resistant to only a single antibiotic, 4 (14%) isolates were resistant to two antibiotics, 2 (7%) isolates were resistant to three antibiotics and 2 (7%) isolates from site D were resistant to four antibiotics. Twenty-one percent (21%) of the *E. coli* isolated was multidrug resistant. The isolated *E. coli* O157:H7 from site D was resistant four of the antibiotics (amoxicillin, sulfamethoxazole, amoxicillin clavulanic acid and ceftriaxone) but susceptible to chloramphenicol, gentamicin and ciprofloxacin. The other *E. coli* O157:H7 isolate from site E was resistant to five antibiotics (sulfamethoxazole, amoxicillin, chloramphenicol and gentamicin and ceftriaxone) but susceptible to amoxicillin clavulanic acid and ciprofloxacin (Supplementary Table 1).

DISCUSSION

Physical parameters: Although, recreational water does not serve as potable water to many individuals across the globe, its quality must meet that of drinking water since many swimmers accidentally drink it during swimming and the high risk of microbial contamination from the environment which poses serious health threat to humans. The study revealed that three out of the five physical parameters (temperature, free chlorine and electrical conductivity) considered were generally within WHO acceptable limits for recreational water (Table 1). The pH and turbidity were the only parameter that fell short of the WHO acceptable limit. This is very serious because lower pH makes the water more acidic hence swimmers may experience burning eyes, itchy skins and their swimming costumes may get torn easily. Also a low pH may result in low effectiveness of the chlorine used in the swimming pool water. A lower pH may even corrode some of the metallic

equipment used in the pool. The turbidity of all the swimming pools recorded high values. This means the water quality is really compromised and may pose health hazards to swimmers. With these critical parameters falling short of the recommended values, it was not surprising therefore, to have high values of fecal coliforms and *E. coli* present all the swimming pools.

In the study, it was observed that the various samples experienced an increase in conductivity from the first sampling to the fourth sampling and this could be attributed to decomposition of organic matter in swimming and external contamination from the surrounding. This vividly shows that the pool attendants do not replace the water regularly as required.

Microbial parameters: The study revealed that the bacteriological quality of swimming pool water of the various swimming pool water samples generally exceeded WHO's limits for recreational water hence making the swimming pool water unwholesome for swimmers. The heterotrophic bacteria counts of the pools were generally within the acceptable standard. However the detection of fecal coliform bacteria and *E. coli* in all the swimming pool water samples indicate fecal contamination of the swimming pool water. Since these organisms are indicator ones, there is a high risk of contracting other pathogenic microorganisms in the swimming pool. This is worrying due to the frequent occurrence of cholera outbreaks in Ghana and the West African sub-region as a whole (Mengel *et al.*, 2014).

The presence of other pathogenic organisms was also made vivid because of the isolation of the pathogenic *E. coli* O157:H7 from two of the swimming pools which are public (site D) and semi-public (site E). This further confirms the outbreak of *E. coli* O157:H7 in swimming pools (Brewster *et al.*, 1994; Keene *et al.*, 1994; Hildebrand *et al.*, 1996; Muller *et al.*, 2001). This really poses a risk to the swimmers because it causes life-threatening diseases such as haemolytic uremic syndrome and other serious complications (Ackman *et al.*, 1997), of which most swimmers are ignorant in the sub-region. These pathogens may enter the swimming pools from infected swimmers or as a result of runoff water which may carry cattle feces (they may naturally harbour *E. coli* O157:H7 in their feces) into the affected pools. The *E. coli* O157:H7 was isolated from site D and C probably because of the high number of swimmers who patronize them. The presence of microbes from all the swimming pools means that the correct dosage of chlorine is not used or these microbes became resistant to the chlorine even if the right dose was used.

Antibiotic susceptibility testing: In this study, seven common antibiotics (ceftriaxone, chloramphenicol, gentamicin, ciprofloxacin, amoxicillin clavulanic acid, sulfamethoxazole and amoxicillin) were used for the susceptibility testing. The susceptibility rate to these antibiotics is generally high (Fig. 1). These susceptibility patterns are, however, contrary to a work done by Saba *et al.* (2013) in Ghana, where isolated *Salmonella* species recorded no resistance to commonly antibiotics used. This means that resistance to commonly used antibiotics by both pathogens and indicator organisms are increasing in Ghana. The isolates obtained in this study, however, had very low resistance to chloramphenicol (21%) and amoxicillin (29%) as compared to *E. coli* isolated from water samples with resistance over 70% for chloramphenicol and amoxicillin in South Africa (Kinge *et al.*, 2010). The resistance of the *E. coli* to ceftriaxone, a third generation cephalosporin which is a drug of choice in Ghana because of its cost, is of concern. This means that very soon, patients may have to opt for more effective and expensive drugs. Resistance to these drugs could easily be transmitted to swimmers who have never taken those antibiotics before because of the risk of swallowing those resistant strains of bacteria during swimming since most swimmers swallow a

bit of the pool water (Dufour *et al.*, 2006). The resistance of these strains could be transferred to other pathogenic microorganisms that may get their way to the swimming pool.

The multiple resistant strains found (21%), in this study is alarming to antibiotic therapy in the country. There is the need for regular testing of swimming pool water in the country, so that precautions can be taken to prevent resistant bugs. In a similar study carried out in Greece (Tirodimos *et al.*, 2010), there were no multiple resistant strains found among *Pseudomonas aeruginosa* isolates from swimming pools. Their isolates recorded only 20% of resistance to the antibiotics used while ours recorded 75% of resistance to the antibiotics used. In a similar study in Ohio, U.S.A., *Pseudomonas aeruginosa* isolated from swimming pools presented 96% rate multiple resistant strains (Lutz and Lee, 2011). Their study had very high multiple resistant rate as compared to present study. The high rate of resistance to sulfamethoxazole (46%) recorded in this study could be attributed to the fact that sulfamethoxazole is found naturally in ground and surface waters due to incomplete treatment of wastewaters (Barber *et al.*, 2009; Underwood *et al.*, 2011) which may find their way into this swimming pools. This may put selective pressure on organisms in the swimming pools hence may cause the higher rate of resistance to sulfamethoxazole.

The two *E. coli* O157:H7 strains found in this study were resistant to more than half of all the antibiotics used and both of them were resistant to ceftriaxone (Supplementary Table 1). This is a very serious revelation because of the ability of these organisms to be resistant to most antibiotics. Outbreaks of *E. coli* O157:H7 and its related strains are not uncommon to the developed countries where they record annual, if not monthly or quarterly outbreaks of these pathogenic strains of *E. coli*. Even though they have better and more effective antibiotics to treat those who contract *E. coli* O157:H7 infections, they still battle with its treatment, lost of productive hours and cost involved as a result of the long stay at the hospitals. When such outbreaks occur in Ghana, it will be disastrous due to the cost involved in treating patients with higher classes of antibiotics which are very costly for the ordinary Ghanaian to afford in their current economic crisis. The greatest concern is that the pathogenic strains and other *E. coli* isolates in this work are extended spectrum beta-lactamase-producing strains (Supplementary Table 1).

CONCLUSION

The results of this study revealed that the physicochemical parameters of the sampled swimming pool water samples were of standards (temperature, electrical conductivity and free chlorine) except the pH and turbidity values which were below standards set by WHO. Fecal coliform and *E. coli* recorded figures that exceeded the WHO guidelines for recreational waters in all the swimming pools. We also isolated two pathogenic and infectious strains of *E. coli* O157:H7 from two of the public swimming pools. Although the general resistances of the isolated organisms were low against the antibiotics used, there were resistant to clinically relevant antibiotics which pose a risk to swimmers and public healthcare in Ghana. There is therefore an urgent need for the authorities in Ghana to ensure that standards are maintained at the various swimming pools through regular surveillance checks and strict enforcement of regulations especially at public swimming pools.

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Supplementary Table 1: Antibiotic susceptibility test results of all the isolates during the four sampling periods

								Eucast break point (mm)	

Antibiotic disk	A1	B1	C1	D1	E1	F1	G1	S _≥	R <
Ceftriaxone (30 µg)	30	26	21	16	19	29	30	23	20
Chloramphenicol (30 µg)	30	20	19	14	10	26	25	17	17
Gentamicin (10 µg)	24	22	20	19	20	23	23	17	14
Ciprofloxacin (5 µg)	28	30	30	28	33	33	33	22	19
Amoxicillin clavulanate (20/10 µg)	6	28	21	20	6	6	6	19	19
Sulphamethoxazole (25 µg)	30	6	6	6	6	6	6	16	13
Amoxicillin (25 µg)	15	17	16	13	15	17	16	14	14
								Eucast break point (mm)	

Antibiotic disk	A2	B2	C2	D2	E2	F2	G2	S _≥	R <
Ceftriaxone (30 µg)	30	23	26	17	30	21	30	23	20
Chloramphenicol (30 µg)	25	25	18	12	22	26	24	17	17
Gentamicin (10 µg)	18	20	22	23	20	21	21	17	14
Ciprofloxacin (5 µg)	16	33	32	30	33	30	30	22	19
Amoxicillin clavulanate (20/10 µg)	20	25	20	20	26	20	20	19	19
Sulphamethoxazole (25 µg)	6	6	6	6	6	6	30	16	13
Amoxicillin (25 µg)	14	15	16	17	15	15	21	14	14
								Eucast break point (mm)	

Antibiotic disk	A3	B3	C3	D3	E3	F3	G3	S _≥	R <
Ceftriaxone (30 µg)	29	27	32	17	12	30	32	23	20
Chloramphenicol (30 µg)	26	26	27	12	20	32	30	17	17
Gentamicin (10 µg)	20	19	21	16	17	20	33	17	14
Ciprofloxacin (5 µg)	30	25	30	29	18	29	32	22	19
Amoxicillin clavulanate (20/10 µg)	20	21	24	22	27	31	28	19	19
Sulphamethoxazole (25 µg)	31	29	21	6	30	34	33	16	13
Amoxicillin (25 µg)	20	18	15	14	15	17	19	14	14
								Eucast break point (mm)	

Antibiotic disk	A4	B4	C4	D4	E4	F4	G4	S _≥	R <
Ceftriaxone (30 µg)	29	30	30	15	19	28	30	23	20
Chloramphenicol (30 µg)	30	28	28	9	28	16	29	17	17
Gentamicin (10 µg)	21	19	27	13	20	18	20	17	14
Ciprofloxacin (5 µg)	29	28	30	24	18	28	20	22	19
Amoxicillin clavulanate (20/10 µg)	22	21	24	30	29	22	22	19	19
Sulphamethoxazole (25 µg)	29	28	28	22	26	29	30	16	13
Amoxicillin (AMX)	14	12	14	12	15	16	11	14	14
								Eucast break point (mm)	

Antibiotic disk	<i>E. coli</i> O157:H7 (D1)				<i>E. coli</i> O157:H7 (E1)			S _≥	R <
Ceftriaxone (30 µg)	12				20			23	20
Chloramphenicol (30 µg)	26				16			17	17
Gentamicin (10 µg)	20				12			17	14
Ciprofloxacin (5 µg)	18				31			22	19
Amoxicillin clavulanate (20/10 µg)	16				22			19	19
Sulphamethoxazole (25 µg)	6				6			16	13
Amoxicillin (AMX)	6				13			14	14

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