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ARTICLE

Phylogenetic grouping based on triplex PCR of multiresistant *Escherichia coli* of environmental, human and animal origin

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ABSTRACT: (Phylogenetic grouping based on triplex PCR of multiresistant *Escherichia coli* of environmental, human and animal origin). *Escherichia coli* is widely used as a biological indicator of faecal contamination due to its ubiquity in faecal material, however, it may have the ability to persist and multiply in environments outside its primary habitat. The genetic substructure in *E. coli* can be determined on the presence/absence of the the genes *chuA* and *yjaA* and a DNA fragment TspE4.C2 based on this method the strains could be assigned to the phylogroups A, B1, B2 or D. This study aimed to carry out phylogenetic affiliation of multiresistant *E. coli* strains from environmental, animal and human samples using the triplex PCR method. Animal and human-origin isolates were associated with a broader multiresistant profile (7 to 13 antimicrobials) and to Extended-Spectrum Beta-Lactamase (ESBL) production. Phylogroup determination demonstrated that B1 (49%) and A (34%) phylogroups were the most prevalent; D (11%) and B2 (6%) were less representative. Phylogroups A and B1 were also related to a broader multiresistant profile. According to the data obtained, the isolates in this study, even the enviromental ones, were associated with human and animal commensal microbiota and not to strains responsible for extra-intestinal infections and had previously been exposed to broad-spectrum antimicrobials.
Keywords: *Escherichia coli,* phylogroup, antimicrobial resistance, poultry, swine, water.

RESUMO: (Grupamento filogenético baseado em PCR triplex de *Escherichia coli* multiresistentes de origem ambiental, humana e animal). A bactéria *Escherichia coli* é largamente utilizada como indicador biológico de contaminação fecal devido à sua ubiquidade em fezes. No entanto, possui também a capacidade de persistir e multiplicar em ambientes fora do seu habitat primário. A subestrutura genética de *E. coli* pode ser determinada através de presença/ausência dos genes *chuA* e *yjaA* e do fragmento de DNA TspE4.C2, com base nesta metodologia cepas podem ser classificadas como pertencentes aos filogrupos A, B1, B2 ou D. Este estudo teve como objetivo realizar a determinação filogenética através do método de PCR triplex de cepas de *E. coli* multirresistentes provenientes de amostras ambientais, animais e humanas. As cepas de origem animal e humana foram associados a perfis de multirresistencia mais amplos (7 a 13 antimicrobianos) e à produção de beta-lactamases de espectro estendido (ESBL). A determinação filogenética demonstrou que os filogrupos B1 (49%) e A (34%) foram os mais prevalentes e os filogrupos D (11%) e B2 (6%), os menos representativos. Os filogrupos A e B1, também foram os mais relacionados a ampla multiresistencia. Os resultados indicaram que todos isolados deste estudo, inclusive os de origem ambiental, são associados a microbiota comensal de humanos e animais e não cepas responsáveis por infecções extraintestinais e que as populações de *E. coli* analisadas sofreram prévia exposição a antimicrobianos de amplo espectro.
Palavras-chave: *Escherichia coli*, filogrupo, resistência antimicrobiana, aves, suínos, água.

INTRODUCTION

The primary habitat of *Escherichia coli* is the intestinal tract of hot blooded animals, and as a singular microorganism in the gastrointestinal microbiota of these animals, it helps their hosts in the breakdown of various molecules (Welch *et al.* 2006). Even in a commensal relationship with the other regular microbiota of the gut, some *E. coli* strains can cause gut and outer-gut infections in a wide variety of hosts, including human beings. The virulence factors shared by many *Escherichia* strains are associated with their opportunist pathogenicity (Yingst *et al.* 2006, Le Gall *et al.* 2007, Clermont *et al.* 2011).

The life cycle of enteric bacteria such as *E. coli*, can involve transition among distinct environments. Some species are restricted to one habitat and others are more widespread, with the ability to live in soil, plants and superficial water (Ratajczak *et al.* 2010, Liang *et al.* 2011). Due to its ubiquity in intestinal tract, *E. coli* is widely used in the evaluation of the microbiological quality of food and water, acting as a key index of faecal contamination and the possible presence of gut pathogenic bacteria. The traits that make *E. coli* one of the best indicators of faecal contamination have been questioned, because studies have shown that some strains of *E. coli* can persist and multiply in the outer environment of the host, in secondary habitats, even in absence of faecal contamination (Power *et al.* 2005, Ishii *et al.* 2006, Luo *et al.* 2011, Pachepsky & Shelton 2011, Moreira *et al.* 2012).

Microorganisms adapted to secondary habitats can also carry genetic factors related to multidrug resistance. The rise of resistant microorganisms, in variable quantities, either in human or animal environments is

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a consequence of the increased use of antimicrobials (Alonso *et al.* 2001, Wright *et al.* 2010). Continuous exposure to antimicrobials has created selective pressure in the commensal microbiota of humans and animals, leading to changes in the drug resistance patterns of these microorganisms, which reflect the previous exposure. These changes can transform these microorganisms into potential reservoirs of resistance genes, facilitating their propagation. Increased resistance to antimicrobials has become a public health concern all over the world, since large spectra antimicrobials, such as β- lactams, are generally associated with multiresistance phenomena (Aminov, 2009).

Particular genotypic and phenotypic characteristics can be related to antimicrobial resistance and the permanence of strains of *E. coli* in secondary habitats. The existence of distinct phylogroups or 'subspecies' of *E. coli* were already known and four phylogenetic groups: A, B1, B2 and D were well characterised (Ochman & Selander 1984, Selander *et al.* 1987). The phylogrouping method based on a triplex PCR (Clermont *et al.* 2000) uses the combination of two genes (*chuA*, the outer-membrane hemin receptor gene, and *yjaA*, which encodes an uncharacterized protein) and a DNA fragment that has been recently identified as part of a putative lipase esterase gene. The strains of these phylogenetic groups differ among themselves in several aspects, such as phenotypic characteristics, antimicrobial resistance profile, the growth rates when related to different temperatures and the distribution of genes, which confers virulence factors on the strains, allowing to some of them a successful extra-intestinal pathogenic life style (Tenaillon *et al.* 2010, Alm *et al.* 2011).

The strains that belong to phylogroups A and B1 are highly adapted to vertebrate animals, where phylogroup A is more associated to humans and phylogroup B1 to animals (Duriez *et al.* 2001, Gordon & Cowling 2003, Skurnik *et al.* 2008). Phylogroup B2 is more associated with strains involved in extraintestinal infections, as is phylogroup D, albeit to a lesser extent (Girardeou *et al.* 2005, Da Silva & Mendonça 2012, Johnson *et al.* 2012). Studies have also concluded that strains of phylogroup B1 may have the ability to persist in aquatic environments and that phylogroups B2 and D are even less frequent in the environment when compared to phylogroups A and B1 (Walk *et al.* 2007, Gordon & Cowling 2003, Ratajczak *et al.* 2010).

Much can be learned about the characteristics of unknown strains of this species through determination of the phylogenetic group. With the importance, in terms of public health, of using *E. coli* as a faecal indicator, added to the emergence of antimicrobial multiresistant strains, and their ability to persist outside the common primary habitat of *E. coli*, this study analysed the frequency of phylogroups among *E. coli* multiresistant isolates obtained from different origins (water, human and animal) using the triplex PCR method.

MATERIALS AND METHODS

Bacterial strains

Forty seven multiresistant environmental-origin *E. coli* isolates were obtained from water samples from Patos Lagoon located at Rio Grande do Sul state (RS) in southern Brazil, collected between autumn 2007 and summer 2008 in eight geographically distinct locations (Canal *et al.*,2016). Animal-origin isolates were obtained from avian and swine faeces sent to the laboratory for isolation of *E. coli* in the period 2011-2013. Faeces samples were suspended and diluted 10^{-1} to 10^{-4} in 0.1% peptone water, homogenised and spread on MacConkey Agar (Himedia). After incubation (24h at 37 °C), intensely pink coloured colonies were selected and re-isolated in Eosin Methylene Blue Agar (EMB - Himedia). The purity of characteristic colonies was checked by Gram staining and biochemical tests (IMViC, hydrogen sulphide gas production (H_2S) and D-sorbitol fermentation) for genus and species confirmation (Winn, 2006). Human-origin isolates were collected at the SANI Group Clinical Laboratory/Passo Fundo, RS, Brazil in 2013. They originated from urine samples of both outpatients and inpatients, and isolated in Sheep Blood Agar 5% and MacConkey Agar. Isolates arrived at the laboratory in Stuart Transport Medium and were re-isolated in EMB Agar (Himedia). After incubation (24 to 48 h at 37 °C), Gram staining of the characteristic colonies was performed, as well as confirmation of genus and species by biochemical tests. All isolates were stored in Brain Heart Infusion Broth (BHI - Himedia) with 15% glycerol at -20 °C, recovered in BHI broth (24 h at 37 ºC), and the purity of cultures always checked by isolation on Tryptone Soy Agar (TSA-Himedia) and Gram stain.

Antimicrobial susceptibility profile of the strains

The antimicrobial susceptibility profile was evaluated using the disk diffusion method in Mueller-Hinton agar (Himedia) (CLSI 2015). All isolates were tested for 15 antimicrobials: ampicillin (10µg; Oxoid), amoxicillin-clavulanic acid (20/10 µg; Oxoid), aztreonam (30µg; Oxoid), imipenem (30 µg; Oxoid), cefoxitin (30µg; Oxoid), ceftazidime (30 µg; Oxoid), cephotaxime (30µg; Oxoid), cefepime (30 µg; Oxoid), piperacillin-tazobactam (100/10µg; Oxoid), amikacin (30µg; Oxoid), gentamicin (30 µg; Oxoid), chloramphenicol (30 µg; Oxoid), sulphamethoxazole-trimethoprim (23.75/1.25 µg; Oxoid), norfloxacin (10 µg; Oxoid) and tetracycline (30 µg; Oxoid), and were considered multiresistant if they were resistant to three or more different classes of antimicrobials. In order to test extended-spectrum β-lactamase (ESBL) production, the disk approximation method was applied, and ESBLs were considered positive-phenotype when the inhibition zone around any of the antimicrobial disks (Ceftazidime, Cephotaxime, Cefepime) was enhanced on the side of the Amoxicillin-Clavulanic acid disk, resulting in a characteristically deformation at the inhibition halo referred to as ghost zone (Garrec *et al.* 2011).

Determination of E. coli phylogroups

DNA extraction was performed as described by Misbah *et al.* (2005), with some modifications. DNA quality was checked via amplification of a 16S rRNA gene fragment, using the oligonucleotides 8F (5-AGAGTTTGATCCTGGATAAG-3') and 926R (5'–CCGTCAATTCCTTTRAGTTT-3') (Liu *et al.* 1997) under the following amplification conditions: one initial cycle of denaturation at 95 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 49.2 °C for 1 minute, and extension at 72 °C for 1 minute, and a final cycle extension at 72 °C for 5 minutes.

Phylogenetic determination of isolates was performed as described by Clermont *et al.* (2000). Three sets of oligonucleotides were used: *chuA* (ChuA1 5'–GACG AACCAACGGTCAGGAT–3'/ChuA2 5'-TGCCGCCAGTACCAAAGACA–3'); *yjaA* (YjaA15'–TGAAGTGTCAGGAGACGCTG–3'/YjaA25'–ATGGAGAATGCGTTCCTCAAC–3') and TspE4.C2 (TspE4C2.15'–GAGTAATGTCGGGGCATTCA–3'/ TspE4C2.25'– CGCGCCAACAAAGTATTACG–3'). Conditions of amplification were: an initial cycle of denaturation at 95 °C for 5 minutes followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing ranging from 55 °C to 60 °C for 1 minute (TspE4.C2 - 55 °C, *chuA* 58 °C, *yjaA* 60 °C) and extension at 72 °C for 1 minute, plus a final extension cycle at 72 °C for 10 minutes. Positive control (*E. coli* ATCC 25922) known to be positive were used for the three kinds of amplification. PCR products were sized as 152 bp (TspE4. C2), 279 bp (*chuA*) and 211 bp (*yjaA*), respectively. The combination of PCR products obtained allowed the *E. coli* isolates to be classified into one of the four major *E. coli* phylogroups: A, B1, B2 and D.

RESULTS

Antimicrobial susceptibility profile

Based on their resistance profile a total of 157 multiresistant *E. coli* strains were selected for this study: 32 human origin, 44 porcine origin, 34 avian origin and the 47 environmental origin. Among the antimicrobials tested, ampicillin and tetracycline (around 96%), sulphamethoxazole-trimethoprim (67%), chloramphenicol (65%) and norfloxacin (50%) were those with the highest resistance. Among the beta-lactams, amoxicillin-clavulanic acid, aztreonam and cephotaxime showed around 30% resistance. Resistance to ceftazidime, cefepime, cefoxitin and piperacillin-tazobactam was around 15-18%. Resistance to aminoglycosides and cephalosporins was higher among human and avian isolates and resistance to cephotaxime and cefepime at the avian set was predominant. The highest resistance percentages for chloramphenicol, tetracycline and sulphamethoxazole-trimethoprim antimicrobials were observed among environmental and animal-origin isolates (Table 1).

Low levels of resistance to imipenem was observed only among the human-origin strains and it was associated to 12 antimicrobial profiles. Resistance to piperacillin-tazobactam was observed among strains from all origins but it was associated to multiresistance profiles in human, porcine and avian strains. Environmental strains did not present resistance to the cephalosporin group, to aztreonam and gentamicin. Resistance to the aminoglycosides was observed mainly among animal origin strains (from 14 - 59%), the environmental strains were resistant only to amikacin (4%).

E. coli phylogroups, antimicrobial multi-resistance and production of ESBLs

Of the 157 multiresistant *E. coli* isolates analysed in this study, regardless their origin, phylogroups A (34%) and B1 (49%) were the most prevalent and phylogroups D and B2 were less prevalent, at 11% and 6%, respectively (Table 2). Human-origin isolates were classified in their majority as phylogroup A (47%), as well as porcine-origin isolates (61%). Among environmental and avian-origin isolates, phylogroup B1 was prevalent (68% and 77% respectively).

Table 1. Susceptibility profile of *E. coli* strains.

	Resistance by strain origin (%)			
Antimicrobials	**Human**	**Porcine**	**Avian**	**Environmental**
Ampicillin	97	100	97	96
Amoxicillin - clavulanic acid	56	16	47	21
Piperacillin-tazobactam	34	18	68	0
Aztreonam	41	14	21	4
Imipenem	50	23	59	0
Ceftazidime	31	5	24	0
Cephotaxime	25	11	82	0
Cefepime	19	2	44	0
Cefoxitin	34	0	35	0
Amikacin	37	9	24	4
Gentamicin	3	0	0	0
Chloramphenicol	41	93	50	74
Norfloxacin	56	48	88	6
Tetracycline	91	91	97	100
Trimethoprim-sulphamethoxazole	47	70	65	89

Table 2. Phylogroup distribution among multiresistant *E. coli* strains.

Phylogroups	*E. coli* strains origin				
	Human origin (n=32)	Porcine origin (n=44)	Avian origin (n=34)	Environmental origin (n=47)	Total (n=157)
A	47%	61%	14%	15%	34%
B1	19%	30%	77%	68%	49%
B2	15%	2%	0	6%	6%
D	19%	7%	9%	11%	11%

The multiresistant profile found among the observed phylogenetic groups were heterogeneous. Of the 15 antimicrobials tested, 84 different multiresistant profiles were obtained. Isolates from phylogroups A and B1 were multiresistant to a larger number of antimicrobials, followed by phylogroups D and B2 isolates. There was a higher diversity of multiresistant profiles in isolates from phylogroup A, either porcine origin isolates (17 profiles) or human origin isolates (15 profiles). This variation in multiresistant profiles was also found among B1 phylogroup isolates; most isolates of this phylogroup were of avian (21 profiles) and environmental origin (8 profiles). Some profiles were unique among the isolates, others were found in more than one phylogenetic group; however, no specific profile among the phylogroups was observed. The most common multiresistant profile was ampicillin, chloramphenicol, tetracycline and sulphamethoxazole-trimethoprim. It was noted that in 31 isolates of environmental and porcine origin with this profile, 21 were phylogroup B1 environmental-origin isolates (Table 3).

Among human-origin isolates, resistance to 6 to 13 antimicrobials was most prevalent (59.38%). Among these, phylogroups A and B2 were the most frequent followed by B1 and D2. Resistance from 3 to 5 antimicrobials was observed in 40.62 % of the isolates, also with a predominance of phylogroups A and B1. Among the porcine-origin isolates, 81.81% presented resistance from 3 to 5 antimicrobials and there was a predominance of phylogroup with few D and B2 and only 18.19% showed resistance from 6 to 11 antimicrobials, most of which were either phylogroup A or B1. Resistance from 6 to 11 antimicrobials was frequent among avian-origin isolates (76.47%) and most of the isolates were A and B1. Among environmental isolates, resistance from 4 to -5 antimicrobials was most common (78.72%) and most were phylogroup B1, with 6 belonging to phylogroup A, three to phylogroup B2 and 3 to phylogroup D (Table 3).

Table 3. Most prevalent multiresistant profiles and their distribution among *E. coli* phylogroups.

Most prevalent profiles	*E.coli* strains origin														
	Human (n=32)				Porcine (n=44)				Avian (n=34)			Environmental (n=47)			
	A	B1	B2	D	A	B1	B2	D	A	B1	D	A	B1	B2	D
AMP ATM CTX CPM GEN TET NOR SUT	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
AMP CLO TET NOR SUT	0	0	0	0	1	6	0	0	0	1	0	1	0	0	0
AMP AMC CLO TET SUT	0	0	0	0	0	0	0	0	0	1	0	5	2	0	0
AMP GEN TET NOR SUT	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0
AMP AMI CLO TET	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
AMP CLO TET SUT	0	0	0	0	5	0	0	2	0	0	0	0	21	3	0
AMP CLO TET NOR	0	0	0	0	3	6	0	0	0	1	0	0	0	0	0
CLO TET NOR SUT	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
AMP TET NOR	1	2	1	0	0	1	0	0	0	0	0	0	1	0	0
AMP CLO TET	0	0	0	0	13	1	1	2	0	0	0	0	0	0	0
Multiresistant profiles															
13 antimicrobials	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
11 antimicrobials	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
10 antimicrobials	1	0	1	0	2	0	0	0	0	2	0	0	0	0	0
9 antimicrobials	1	0	1	0	0	0	0	0	0	8	0	0	0	0	0
8 antimicrobials	1	0	0	1	0	1	0	0	1	7	0	0	0	0	0
7 antimicrobials	1	1	1	0	3	1	0	0	0	2	0	0	0	0	0
6 antimicrobials	3	1	1	3	0	0	0	0	2	2	0	0	0	0	0
5 antimicrobials	1	1	0	1	1	2	0	0	2	0	0	6	2	0	0
4 antimicrobials	4	1	0	1	6	6	0	0	0	2	2	0	23	3	3
3 antimicrobials	1	2	2	0	14	3	1	2	0	2	0	1	7	0	2

Antimicrobials: ampicillin (AMP), aztreonam (ATM), amoxicillin - clavulanic acid (AMC), cephotaxime (CTX), cefepime (CPM), gentamicin (GEN), amikacin (AMI), chloramphenicol (CLO), tetracycline (TET) norfloxacin (NOR) and trimethoprim-sulphamethoxazole (SUT).

Table 4. Distribution of phylogroups among *E. coli* strains for ESBL phenotypically positive.

Phylogroup	*E. coli* strains origin		
	Human (n=16)	Porcine (n=8)	Avian (n=28)
A	43%	63%	18%
B1	19%	37%	79%
B2	19%	0	0
D	19%	0	3%

Extended spectrum beta-lactamase (ESBLs) production was observed in 33% of all isolates. ESBL production was not phenotypically detected in only the environmental-origin isolates, it was more frequent among avian (82%) and human-origin (50%) isolates. When related to phylogenetic group, presence of phenotypically positive isolates for ESBL production was more closely associated with A and B1 phylogroups (Table 4).

DISCUSSION

Resistance was higher for tetracyclines, penicillins, quinolones, amphenicols, sulphonamides, aminoglycosides and cephalosporins, which indicates the continued exposure of these populations to a wide variety of antimicrobials, which favours selection of resistant strains and the dissemination of genetic elements related to this in different habitats.

Koczura *et al.* (2012) studying environmental and human origin of *E. coli* isolates, observed a high rate of resistance to some of the antimicrobials tested, with multi-resistance more often associated to human-origin isolates, compared to environmental, highlighting resistance to aminoglycosides, cephalosporins and aztreonam. The influence of environmental selective pressure was pointed by the authors, since the microorganisms isolated were from inpatients and from environments related to wastewater discharge in a treatment plant. Yan-yan *et al.* (2013) found 100% resistance to some third-generation cephalosporins and resistance at a high level to fluoroquinolones among environmental, porcine, healthy and hospitalized patient human origin *E. coli* isolates, which indicated the development of resistance mechanisms through continued exposure to antimicrobials. In our study, the cephalosporin-resistance percentage was not high, except for cephotaxime and cefepime in avian-origin isolates (82% and 44% respectively), which reinforces the view that the populations evaluated may have been indiscriminately exposed to these antimicrobials. The data indicates that the overuse of antimicrobials, such as ceftiofur, in poultry can promote cross-resistance to cephalosporins used in medical practice, and contribute to the increased failure of therapies, and the most severe cases of infectious diseases caused by enterobacteria (Dutil *et al.* 2010). Some studies have connected multi-resistance dissemination to the use of antimicrobials in animal production, not only for control of diseases caused by *E. coli*, but also for their prophylactic control, and this is likely to represent a food safety risk to consumers (Gonçalves *et al.* 2010, Drummond & Perecmanis, 2013).

Environmental origin isolates showed a restrict multiresistant profile, as 72% of isolates were resistant to only four antimicrobials. Within these, 66% presented resistance to ampicillin, chloramphenicol, sulphamethoxazole-trimethoprim and tetracycline. In a study carried out in Bangladesh, *E. coli* isolates obtained from the local water supply network presented multi-resistance profiles, where ampicillin and tetracycline were the antimicrobials with higher percentages of resistance, which matches our results (Talukdar *et al.* 2013). Pereira *et al.* (2013), working with *E. coli* isolates from an estuary in southern Brazil, found lower levels of multi-resistance; however, they considered the prevalence of antimicrobial resistance high because of the origin of the isolates, emphasising the occurrence of resistance to antimicrobials used as last resort for infection treatment, which would indicate probable environmental contamination. As isolates in our study were obtained from samples collected near an urban area, the influence of human activity and the presence of forms of dissemination of resistance genes could be related to the multi-resistance profiles obtained.

The results concerning phylogenetic distribution suggested that the strains studied are mostly associated with commensal microbiota of humans and animals, and not higher pathogenic-profile *E. coli* strains; as phylogroups A and B1 are, in general, associated with commensal isolates, whereas B2 and D phylogroups are often associated with extra-intestinal diseases with a greater number of virulence factors (Clermont *et al.* 2000, Duriez *et al.* 2001, Gordon & Cowling 2003, Gordon 2004, Sabaté *et al.* 2006, Bagheri *et al.* 2014). According to Walk *et al.* (2007), B2 and D phylogroups are not strongly associated with environmental isolates, and there is high prevalence of phylogroup B1 in aquatic environments because many strains of this phylogroup are able to survive there. These results may be related to high genetic variation in the species, because the factors that shape the genetic structure of a microbial population are influenced by host characteristics and the environment to which this organism is exposed (Tenaillon *et al.* 2010).

There was a wide range of multi-resistance profiles associated with phylogenetic classification within the isolates evaluated; it was not possible to associate multiresistance profiles to phylogroups. Perhaps this is connected to the adaptations of microorganisms to the habitat, which allows the most pathogenic strains of *E. coli* to establish associations with commensal strains, providing a transit of resistance genes through genetic elements in microenvironments within the same host and outside it, resulting in dissemination of these factors in the environment (Croxen & Finlay 2010, Sommer & Dantas 2011, Alteri & Mobley 2012).

The multiresistant (7 to 12 antimicrobials) avian origin isolates were almost all phylogroup B1 and one isolate was phylogroup D (resistant to 11 antimicrobials out of the 15 tested). All were resistant to cephotaxime,

sometimes combined with ceftazidime and cefoxitin, as mentioned before, the use of ceftiofur in poultry favours the establishment of these multiresistant strains. This finding represents an additional concern, although the majority of the isolates were considered commensal *E. coli* and may occasionally cause infections in predisposed humans, they may also carry a variety of resistance and virulence determinants that increase their potential for causing disease in humans. Another concern is that these strains could potentially become reservoirs of these genes for human pathogens, including *Salmonella* and others. Poppe *et al.* (2005) have demonstrated experimentally the acquisition of resistance to extended-spectrum cephalosporins by *Salmonella* serovar Newport from *E. coli* strains by conjugation in poultry intestinal tracts.

The prevalence of isolates that were phenotypically positive for beta-lactamase was not high. When related to phylogenetic distribution and to ESBL production, the antimicrobial susceptibility profile supported the results obtained, since the percentage of resistance to amoxicillin-clavulanic acid and piperacillin-tazobactam were higher in both human-origin isolates and avian origin isolates. Conversely, aztreonam, imipenem, ceftazidime, cephotaxime and cefoxitin susceptibility was 100% among environmental isolates, which have not presented this phenotype. Many studies have shown that *E. coli* isolated from clinical samples and of avian origin carried several beta-lactamases classes (Machado *et al.* 2008, Oteo *et al.* 2010, Bagheri *et al.* 2014). Another study that compares data from 2001, 2006 and 2010 found that the prevalence of *E. coli* producers of ESBL had decreased; which is related to a decreasing use of antimicrobials in the population over that period, and led the authors to reason, as in this study, that exposure of populations to antimicrobials is likely to impact dissemination rates of *E. coli* that produce penicillinase in the community (Woerther *et al.* 2013).

Studies have associated human *E. coli* ESBLs producers mainly with B2 and D phylogroups and with lower percentages of A and B1 phylogroups (Johnson *et al.* 2012, Brolund *et al.* 2013). In our study, the frequent presence of ESBL producers belonging to the A phylogroup was observed. These results may be related to the origin of the isolates, as they were isolated from samples of healthy people. Another hypothesis is that due to the prevalence of B2 phylogroup isolates in this study being low (6%), the occurrence of isolates classified in this phylogroup and ESBL producers, was consequently low compared to other phylogroups. It is likely that, in a study of equivalent incidences among phylogroups, more ESBL producers could be found in the B2 phylogroup.

When compared to human origin and avian origin strains, the porcine origin strains studied were positive for these penicillinases at a lower level, reflecting their higher susceptibility to cephalosporins and piperacillin-tazobactam. Other studies that evaluated *E. coli* multiresistant to antimicrobials isolated from swine and other mammals, found low percentages of positivity to ESBL production, inferring a range of incidence of this phenotype among the populations (Gonçalves *et al.* 2010, Wagner *et al.* 2014).

CONCLUSIONS

This study provided evidence that the phylogroups proposed by Clermont *et al.* (2000) are disseminated among different sources of isolation and multiresistant *E. coli* strains demonstrated a considerable level of genetic diversity. Phylogroups A and B1 were the most frequent and the most related to multi-resistance; and the high prevalence of the phylogroup B1 in the environmental origin set of isolates can be related to the sample collection location (urban area) or the ability of determined strains associated with this phylogroup to survive in secondary habitats in saprophytic form.

The production of extended spectrum beta-lactamases among the phylogroups presented a heterogeneous distribution, was restricted to animal and human-origin isolates, and was prevalent among A and B1 phylogroups, normally associated with commensal isolates. It is a public health concern that these strains could potentially act as a reservoir of ESBL genes for other human pathogens, they may occasionally cause infections in predisposed humans and may be associated with treatment failure, increased risk of infection, more severe outcome and longer duration of illness.

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