VIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE OF ESCHERICHIA COLI ISOLATED FROM URINARY TRACT OF SWINE IN SOUTHERN OF BRAZIL

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Submitted: September 03, 2007; Returned to authors for corrections: January 08, 2008; Approved: November 05, 2008.

ABSTRACT

The present study determined the molecular and resistance patterns of E. coli isolates from urinary tract of swine in Southern of Brazil. Molecular characterization of urinary vesicle samples was performed by PCR detection of virulence factors from ETEC, STEC and UPEC. From a total of 82 E. coli isolates, 34 (38.63%) harbored one or more virulence factors. The frequency of virulence factors genes detected by PCR were: pap (10.97%), hlyA (10.97%), iha (9.75%), sta (7.31%), sfa (6.09%), f4 (4.87%), f5 (4.87%), stb (4.87%), f6 (1.21%) and f41 (1.21%). Isolates were resistant to penicillin (95.12%), lincomycin (93.9%), erythromycin (92.68%), tetracycline (90.24%), amoxicillin (82.92%), ampicillin (74.39%), josamycin (79.26%), norfloxacin (58.53%), enrofloxacin (57.31%), gentamicin (39.02%), neomycin (37.8%), apramycin (30.48%), colistine (30.48%) and cefalexin (6.09%). A number of 32 (39.02%) E. coli isolates harbored plasmids.

Key words: E. coli, plasmids, virulence factors, antimicrobial resistance, swine

The urinary tract infection (UTI) is defined by the presence of bacteria in the urinary tract (22). In humans, the disease is associated to many virulence factors present in the uropathogenic E. coli (UPEC), including haemolysin, aerobactin, adhesins, serum resistance, cytotoxic necrotizing factor (CNF), capsule production and uropathogenic-specific protein (14,15,21). E. coli adhesion to host cells is important to bacteria infection and persistence in urinary fluxes (6,10). The genes involved in biosynthesis of fimbria and adhesins present in UPEC are organized in operons denominated pap and sfa, coding for P and S fimbrial adhesins (2,3,24). IRGA homologue adhesin (IHA) is an outer membrane protein (OMP) found mainly in UPEC being also involved in adherence (13). Two toxin types are associated to UPEC: The alfa-haemolysin (HLY) and cytotoxic necrotizing factor 1 (CNF 1) are involved in host cell destruction necessary to bacteria persistence in urinary tract (4,8). Toxins from enterotoxigenic E. coli were described in swine urinary E. coli isolates as heat-labile toxin (LT) and vero toxin (VT) (3).

The antibiotic therapy may select resistant bacteria (5,22). The resistance to antimicrobial drugs may be carried by plasmids, as well as chromosomal mutations that occur spontaneously (9). Multi-drug resistance have been reported in human and swine E. coli isolated from urinary tract (4,11). The purpose of the present study was to determinate the pathotype, the plasmidial DNA content and the patterns of resistance to antimicrobial drugs in E. coli isolates from swine females with UTI.

Eighty two E. coli strains were isolated from sows from thirty breeding farms. Animals were considered with urinary infection according to microbiologic and urinary physic chemical patterns (22). The E. coli were isolated from urinary vesicle and urine samples collected from swine breeding farms in Southern of Brazil. One putative E. coli colony was identified by morphology and biochemical tests according to Quinn et al.

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The Kirby-Bauer disc diffusion test (17) was used and the following drugs were tested: amoxicillin (10 μg), ampicillin (10 μg), tetracycline (30 μg), norfloxacin (10 μg), enrofloxacin (5 μg), cefalexin (30 μg), neomycin (30 μg) gentamicin (30 μg), penicillin (10 μg), lincomycin (2 μg), erythromycin (15 μg), apramycin (15 μg), josamycin (30 μg), and colistin (10 μg).

The E. coli isolates were characterized by multiplex PCR for fimbrial and toxin genotypification by using the amplification of the following regions: sta, stb, stx, cnf, hly, lt, f4, f5, f6, f41, f18, bfp, eae, sfa, pap, iha and usp. The primers and PCR conditions were previously described (1,2,4,7,12,15,16). Amplicons identities were confirmed by sequencing (Amersham Pharmacia Biotech). Plasmidial DNA extraction from E. coli isolates was performed by alkaline lysis, as previously described (4).

From E. coli isolates, 34 (38.63%) harbored one or more virulence factor revealed by PCR amplification. UPEC were found in 14.63% (12/82), ETEC in 15.85% (13/82) and in 10.97% (9/82) were amplified virulence factors of both, ETEC and UPEC. Brito et al. (4) described the occurrence of ETEC and UPEC in swine with UTI. According to Russo and Jonhson (19) the current E. coli pathotype classification are performed by a combination of virulence traits, and not by genetically source. ETEC were reported in swine urinary strains suggesting the ascending intestinal origin of UTI (4,23). The pathogenesis of urinary tract infections depends of the E. coli skills to adhere, persist and multiply in the host (5). The genes involved in bacteria adherence detected in our study were pap (10.97%), iha (9.75%), sfa (6.09%), f4 (4.87%), f5 (4.87%), f6 (1.21%) and f41 (1.21%). The pap, iha and sfa elements are reported as important to adhesion of UPEC (13,24). Pap gene was found in 54.8% of Brazilian UPEC isolates studied by Brito et al. (4), although in our study, the frequency of this gene was lower (10.97%). According to Brito (4) sfa, Bfp and sfa adhesins were not found in UPEC isolates from Brazil. In our study we found sfa in 6.09% E. coli isolates. The f4 and f5 fimbriae genes were amplified alone or in association with virulence factors typical of UPEC.

The presence of haemolysis in blood agar was observed in four isolates, although the hlyA gene was detected by PCR in nine isolates. This difference may be associated to silent expression or mutation in hly genes in E. coli (20,21). Brito et al. (4) reported the presence of 25.8% of haemolytic UPEC isolates. In our study lt, sta and stb toxins genes commonly found in ETEC were detected, respectively, in 8.53%, 7.31% and 4.87% of E. coli isolates. Lt was previously described in swine UPEC by Brito et al. (4).

The presence of usp gene in dog and cat E. coli isolates permitted the proposition of the role these animals as an alternative reservoir for human UTI (14,15). In contrast, we amplified usp in one E. coli isolated and this may suggest genotypic differences among swine and human urinary E. coli isolates.

The antimicrobial resistances of UPEC isolates are presented in Fig. 1. Brito et al. (4) report a higher resistance of swine UPEC to tetracycline and ampicillin. In swine UPEC 95.12% (78/82) were resistant to four or more antimicrobial groups. The more frequent patterns of resistance were to beta lactam, lincomicin, tetracycline, quinolone, macrolide, aminoglycoside and polymyxin groups (data not shown). Plasmids and other genetic elements, like integrons, may be encountered in UPEC and are associated to coding virulence factors and MDR (4,5,18). In our study plasmids were found in 39.02% (32/82) of E. coli isolates.

Some E. coli isolates maintain virulence factors of both ETEC and UPEC simultaneously, suggesting the elevated genetic relationship between urinary and intestinal strains. Multi-drug resistance was widely found in swine urinary isolates.

![Figure 1. Antimicrobial drugs resistance patterns of urinary E. coli isolates from swine in Southern of Brazil. Penicillin (Pen), Lincomycin (Lin), Erythromycin (Eri), Tetracycline (Tet), Amoxicillin (Amo), Josamycin (Jos), Ampicillin (Amp), Norfloxacin (Nor), Enrofloxacin (Eno), Gentamicin (Gen), Neomycin (Neo), Apramycin (Apr), Colistin (Col) and Cefalexin (Cef).](image-url)
Os isolados foram resistentes à penicilina (95,12%), lincomicina (93,9%), eritromicina (92,68%), tetraciclina (90,24%), amoxicilina (82,92%), ampicilina (74,39%), josamicina (79,26%), norfloxacina (58,53%), enrofloxacina (57,31%), gentamicina (39,02%), neomicina (37,8%), apramicina (30,48%), colistina (30,48%) e cefalexina (6,09%). Trinta e dois (39,02%) isolados de *E. coli* continham plasmídeos.

**Palavras chave:** *E. coli*, plasmídeos, fatores de virulência, resistência antimicrobiana, suínos.

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