

IN VITRO SUSCEPTIBILITY TO 11 ANTIMICROBIAL AGENTS OF *Escherichia coli* ISOLATED FROM DIARRHEIC AND MASTITIC CATTLE IN SÃO PAULO STATE, BRAZIL

(*SENSIBILIDADE IN VITRO A 11 AGENTES ANTIMICROBIANOS DE CEPAS DE
Escherichia coli ISOLADAS DE GADO DIARREICO E MASTITICO COLHIDAS NO
ESTADO DE SÃO PAULO, BRASIL*)

(*SENSIBILIDAD IN VITRO A 11 AGENTES ANTIMICROBIANOS DE CEPAS DE Escherichia coli
AISLADAS DE GANADO DIARREICO Y MASTÍTICO COLECTADAS EN EL ESTADO DE
SÃO PAULO, BRASIL*)

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SUMMARY

Emergence of bacterial antimicrobial resistance has become a serious worldwide problem. Antimicrobial susceptibility testing was performed on a total of 150 *Escherichia coli* isolates from diarrheic, mastitic and control cattle. Among the 11 antimicrobial agents tested, resistance was most frequently found for ampicillin, streptomycin and tetracycline in isolates from the diarrheic and mastitic groups. The isolates from the control group were susceptible to all agents tested. Multidrug resistance was found among 30.0% of the isolates from the diarrheic group and among 14.0% from the mastitic group. The potential of the transfer of antimicrobial resistance by enteric bacteria from animals used as food source to human populations is a cause of concern. Surveillance is an essential part of a policy of rational use of antimicrobial agents and should lead to regular and continuous monitoring of the antimicrobial bacterial resistance to avoid its increase among animal faecal flora.

KEY-WORDS: *Escherichia coli*. Antimicrobial resistance. Bovines. Surveillance.

RESUMO

A existência de resistência aos agentes antimicrobianos entre as bactérias tem-se revelado um sério problema o qual afeta o mundo inteiro. Teste de sensibilidade a antimicrobianos foi realizado em 150 cepas de *Escherichia coli* provenientes de gado diarreico, mastítico e saudável. Entre os 11 agentes antimicrobianos testados, a resistência mais frequentemente encontrada foi para ampicilina, estreptomicina e tetraciclina, entre as cepas provenientes dos grupos diarreico e mastítico. As cepas provenientes do grupo controle se mostraram sensíveis a todos os agentes antimicrobianos testados. Multiresistência a dois ou mais agentes antimicrobianos foi mostrada em 30,0% das cepas provenientes do grupo diarreico e 14,0% das cepas provenientes do grupo mastítico. A potencialidade de transferência de resistência a antimicrobianos das bactérias entéricas dos animais para as populações humanas representa um motivo de preocupação. O levantamento de informações é fundamental na utilização racional de agentes antimicrobianos e deve possuir um monitoramento regular e contínuo, evitando o aumento da resistência a antimicrobianos na flora fecal.

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PALAVRAS-CHAVE: *Escherichia coli*. Resistência a antimicrobianos. Bovinos. Levantamento epidemiológico.

RESUMEN

La existencia de resistencia a los agentes antimicrobianos entre las bacterias se ha revelado como un serio problema que afecta el mundo entero. El teste de sensibilidad a antimicrobianos fue realizado en 150 cepas de *Escherichia coli* provenientes de ganado diarreico, mastítico y saludable. Entre los 11 agentes antimicrobianos testados la resistencia más frecuentemente encontrada fue para la ampicilina, estreptomocina y tetraciclina, entre las cepas provenientes de los grupos diarreico y mastítico. Las cepas provenientes del grupo control se mostraron sensibles a todos los agentes antimicrobianos testados. Multiresistencia a dos o más agentes antimicrobianos fue mostrada en 30,0% de las cepas provenientes del grupo diarreico y 14,0% de las provenientes del grupo mastítico. La potencialidad de transferencia de resistencia a antimicrobianos de las bacterias entéricas de los animales para las poblaciones humanas representa un motivo de preocupación. El levantamiento de informaciones es fundamental en la utilización racional de agentes antimicrobianos y debe haber monitoreo regular y continuo, evitando el aumento de la resistencia a antimicrobianos en la flora fecal.

PALABRAS-CLAVE: *Escherichia coli*. Resistencia a antimicrobianos. Bovinos. Levantamiento epidemiológico.

INTRODUCTION

The emergence and dissemination of antimicrobial resistance in bacteria, a serious worldwide problem has been well documented (COHEN, 2000). Selective pressure favoring increased antimicrobial-resistant phenotypes occurs whenever antimicrobials are used, in clinical medicine, in preventing disease and in promoting growth in animal husbandry. As a consequence, antimicrobial-resistant bacteria are selected, thereby posing a critical public health threat by reducing the efficacy of antimicrobial treatment (TEUBER, 2001, LEVY, 2002, SCHROEDER et al., 2002).

The prevalence and degree of antibiotic resistance found in bacteria of the fecal flora of animals are considered good indicators of the selective pressure of antibiotic usage, they correlate with the amount and types of antimicrobial agents consumed by animal populations (VAN DEN BOGAARD e STOBLELINGH, 2000, VANDEN BOGAARD et al., 2000).

Many studies on antimicrobial resistance rates of animal bacteria mostly *Escherichia coli* have been published during the last few years (AARESTRUP et al., 1998, HOYLE et al., 2004). A number of studies on *E. coli* resistance have become available, but most of them show restrictions in one way or another. Current strategies to monitor the presence of antibiotic resistant bacteria in animals used as a food sources, target mainly resistance in clinical specimens, and only periodically involve cross-sectional evaluation of resistance in faecal flora on a large scale (CAPRIOLI et al., 2000, GUN et al., 2003). However, such surveys do not provide any information about the dynamics of antibiotic resistance in the normal flora and the possible interaction of diseased cows with healthier ones of the same herd. In this study we therefore investigated the antibiotic resistance of *E. coli* isolates

from only one farm, to evaluate the transmission of resistance within a herd and its potential danger to human health.

MATERIAL AND METHODS

BACTERIAL ISOLATES

A total of 150 *E. coli* strains were isolated from fifty mastitic, fifty diarrheic and fifty control animals living at a cattle farm in São Paulo State, Brazil, between January and December of 2004. Fecal samples from diarrheic and health cattle were taken rectally, using sterile cotton tipped swabs, and transported within 2 h to the laboratory. Cows suspected of having mastitis were evaluated by the California Mastitis Test (CMT) according to the method proposed by Schalm e Noorlander (1957) and placed on a scale ranging from 1-5 (KLASTRUP, 1975). For milk sampling, teat ends were cleaned with (70%) alcohol; swabs moistened and allowed to dry. After discarding the first few milk streams, (2-4mL) samples were collected into sterile 10 mL glass flasks and submitted to CMT. Positive samples were refrigerated to about 4 °C and dispatched to the laboratory without delay. *E. coli* culture and isolation were performed according to standard microbiological procedures. Briefly, a fecal sample on a cotton tipped swab or a loop full sample of milk was streaked for isolation directly on Mac Conkey agar dishes and incubated for 24 h at 37°C. Only one lactose-positive colony from each sample was picked and re-streaked onto Mac Conkey agar and incubated for a further 24 h at 37°C. Biochemical confirmation of the strains was performed and *E. coli* was defined as oxidase negative, indole positive, Simon's citrate negative, urease negative and hydrogen sulfide negative (KONEMAN et al., 1997).

SUSCEPTIBILITY TESTING

Antimicrobial disk susceptibility tests were performed using the disk diffusion method (BAUER et al., 1966) as recommend by the NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS (NCCLS, 1999). *E. coli* isolates were transferred from the Mac Conkey agar plates to trypticase soy broth (TSB). The cells were allowed to grow for approximately 4-6h at 37 °C, until they exceeded the 0.5 McFarland turbidity standard. NaCl sterile solution (0.85%) was inoculated into the TSB suspension to achieve an optical density corresponding to 0.5 McFarland units. A sterile non-toxic swab was dipped into the adjusted suspension and streaked onto Mueller-Hinton agar plates to form a uniform lawn of bacterial growth. Drug-impregnated disks (CEFAR) were placed on the surface of the agar using a disk dispenser. Eleven antimicrobial agents were selected for the tests: ampicillin (AMP-10µg); amoxicillin/clavulanic acid (AMC-30µg); cephalothin (CEP-30µg); ceftriaxone (CEF-30µg);

tetracycline (TET-10µg); gentamicin (GEN-5µg); streptomycin (S-10µg); amikacin (AMK-30µg); trimethoprin (TMP-16µg); nalidixic acid (NAL-30µg) and ciprofloxacin (CIP-5µg).

RESULTS AND DISCUSSION

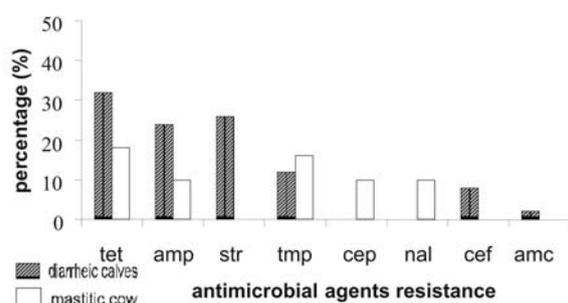
Prevalence and extent of antimicrobial resistance in a population is strongly correlated to antibiotic usage, as selection and dissemination of resistant bacteria are greatly increased by the pressure exerted by these drugs. As a consequence, resistance is most commonly found where there is heavy use of antibiotics and appreciable host to host contact: sites of intensive farming constitute a large reservoir of antibiotic resistant bacteria (MURRAY, 1992). Here resistance is selected and perpetuated not only by the regular veterinary use of antibiotics, but also by the continuous feeding of antibiotics to enhance animal

Table 1 – Patterns and phenotypes of drug resistance amongst 50 strains of *Escherichia coli* isolated from diarrheic cattle and 50 strains isolated from mastitic cattle in Ituverava/SP, Brazil, between January-December 2004.

| PATTERNS | PHENOTYPES | ORIGIN |
|--------------------------|--------------------------|--------------------|
| Resistant to none (78)* | Sensitive (78) | Diarrheic/mastitic |
| Resistant to 2 drugs (8) | amp-tet (1) | Diarrheic |
| | str- tet (3) | Diarrheic |
| | amp-tmp (1) | Diarrheic |
| | cef-tmp (1) | Mastitic |
| | cef-tet (1) | Mastitic |
| | tmp-nal (1) | Mastitic |
| | Resistant to 3 drugs (5) | amp-str-tet (4) |
| amp-str-tmp (1) | | Diarrheic |
| Resistant to 4 drugs (9) | amp-str-tet-tmp (4) | Diarrheic |
| | amp-amc-str-tet (1) | Diarrheic |
| | amp-cef-nal-tmp (4) | Mastitic |

* Number of strains is given in parentheses.

Amc- amoxicillin/clavulanic acid; Amk- amikacin; Amp- ampicillin; Cef- ceftriaxone; Cep- cephalothin; Cip- ciprofloxacin; Gen- gentamicin; Nal- nalidixic acid; Str- streptomycin; Tet- tetracycline; Tmp- trimethoprin.



Amc- amoxicillin/clavulanic acid; Amp- ampicillin; Cef- ceftriaxone; Cep- cephalothin; Nal- nalidixic acid; Str- streptomycin; Tet- tetracycline; Tmp- trimethoprin.

Figure 1- Antimicrobial resistance patterns in *Escherichia coli* isolates from diarrheic and mastitic cattle in Ituverava/SP, Brazil, between January-December 2004.

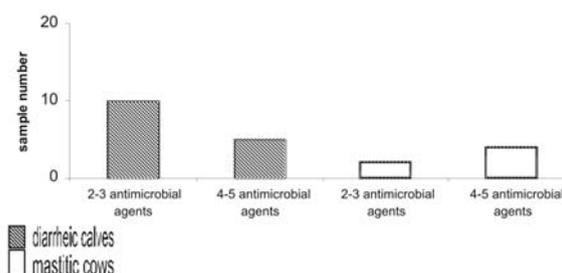


Figure 2- Distribution of multi-drug resistance to 11 antimicrobial drugs among 50 strains of *Escherichia coli* isolated from diarrheic and 50 strains isolated from mastitic cattle, in Ituverava/SP, Brazil, between January-December 2004.

growth (VANDEN BOGAARD et al., 2000, TEUBER, 2001). In this situation, resistant microorganisms are very easily disseminated within units via fecal contact, promoting contamination of the water used by animals or environmental contamination of the soil.

Among the 150 isolates tested, *E. coli* resistance rates of approximately 30% were observed for tetracycline, ampicillin and streptomycin among the isolates from diarrheic cattle, rates of approximately 15% were observed for tetracycline, ampicillin and trimethoprim among the isolates from mastitic cattle, no resistance was observed among isolates from healthy cattle (figure 1). A high level of resistance to these drugs has been shown among *E. coli* isolates from bovine cattle (ADESIYIN e KAMINJOLO, 1982, BEZEK, 1998, ORDEN et al., 2000, ZHAO et al., 2001, WERCKENTHIN et al., 2002, BERGE et al., 2003, GUERRA et al., 2003, LEHTOLAINEN et al., 2003). The discrete level of resistance for these drugs reported here agrees with reports of Langoni et al. (2000), of 13.0% of resistance to tetracycline and of 12.0% to ampicillin; these are very different from those reported by Amaral et al. (1996), namely 92.9% to ampicillin and 71.4% to tetracycline.

Analyses of multidrug-resistance patterns (table 1), showed tetracycline, ampicillin, streptomycin and trimethoprim grouped together in most of these patterns. The four antimicrobial agents are well known to be connected in a resistance gene cluster in *Salmonella* (CLOECKAERT e SCHWARG, 2001). There are hints that a similar situation may exist for *E. coli* (CLOECKAERT et al., 2000, WHITE et al., 2000). However, the genetic basis of cross-resistance of *E. coli* has not been established so far.

The frequency of multidrug resistant bacteria detected in this work (figure 2), is lower than that reported by Orden et al (2000); 76.9% for *E. coli* isolates from Spain and Berger et al. (2003) 74.8% for isolates from the USA, and also for isolates from Brazil, as reported by Amaral et al. (1996), 92.8% and by Moreno et al. (1997), 90.0%.

Knowledge of resistance transfer via plasmids, phages, transposition and “free” DNA has increased considerably since the 1970’s and 1980’s, when such transfer was considered to be uncommon (KNOTHE, 1977). It was then thought that only zoonotic bacteria from animals could infect humans; transfer of resistance genes from animal bacteria to human bacteria was thought to be rare (LACEY, 1980). Presently fear is that antibiotic-resistant bacteria, pathogenic or non-pathogenic to humans, are selected in the intestinal flora of animals, contaminating food of animal origin and transferring their resistance to other bacteria in the human gut (DONNELLY et al., 1996, PIDDOCK, 1996). In this sense, it is alarming that half of all antibiotics produced in the USA are used in animals (TEUBER, 2001), this data reflecting the level of antibiotic resistance in the USA, compared to what

observed in countries with a rigid control of antibiotic usage.

Another aspect to be pointed out is the total susceptibility of *E. coli* isolates from healthy cattle to the 11 antimicrobial agents tested. A very different situation was reported by Berge et al. (2003) who examined 297 calves from 10 farms in California and isolated 5366 *E. coli* comensal strains; according to the authors, multi-resistance to tetracycline, streptomycin and sulfamethoxazole/trimethoprim was detected in 65.0% of the strains; 61.0% also showed resistance to ampicillin.

To conclude, this study describes the level of susceptibility to 11 antimicrobial agents of 150 *E. coli* isolates from mastitic, diarrheic and healthy cattle. The level of resistance detected among the isolates was discrete, but not-negligible. Continued surveillance of *E. coli* collected from animals is important for the identification of emerging antimicrobial resistant phenotypes.

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