ANTIBIOGRAM OF ENTEROTOXIGENIC AND ENTEROHAEMORRHAGIC ESCHERICHIA COLI ISOLATED FROM COWS AND CALVES

P. KHURANA and ASHWANI KUMAR¹
Department of Veterinary Public Health, College of Veterinary Sciences
CCS Haryana Agricultural University, Hisar-125 004

SUMMARY

Antibiograms of 100 isolates of *E. coli* including 22 of enterohaemerrhagic and 37 of enterotoxigenic groups were determined by disc diffusion technique by employing 13 antimicrobial drugs. Sensitivity of enterohaemerrhagic and enterotoxigenic isolates was highest to norfloxacin and ciprofloxacin (94.5-100%) and gentamicin (81.8-83.7%). All the isolates were resistant to tetracycline while moderate to high resistance was shown against polymyxin-B, nitrofurazone, chloramphenicol, cephalexin, cephadroxil and ampicillin. Resistance of these human pathogens to a number of antimicrobial drugs has great public health significance.

Key words: EHEC, ETEC, resistance

Due to indiscriminate use of antibiotics in animal husbandry to treat infections and to improve growth and feed efficiency of animals, antimicrobial resistance has become a public health threat. E. coli is known to be an important cause of diarrhea in man and animals. Five distinct groups including enterohaemorrhagic (EHEC) and enterotoxigenic (ETEC) E. coli causing diarrhea have now been identified and these two groups are responsible for serious disease conditions in humans. These groups of E. coli are present in the gut of man and animals and their environment and can be transmitted to man through the food supply. Keeping in view the public health significance of drug resistance among foodborne pathogens, the present study was undertaken to study the antibiogram of E. coli isolated from cows and calves.

Antibiograms of 100 isolates of *E. coli* including 22 isolates of EHEC and 37 of ETEC, were determined by disc diffusion technique (Bauer *et al.*, 1966). These *E. coli* were previously isolated from milk/ and faeces of cows and calves. The antibiotic discs (Hi-Media) employed were ampicillin (10 mcg), cephadroxil (30 mcg), cephalexin (30 mcg), chloramphenicol (30 mcg), ciprofloxacin (5 mcg), gentamicin (30

mcg), nitrofurazone (100 mcg), norfloxacin, (10 mcg), polymyxin-B (300 units), streptomycin (10 mcg), spectinomycin, (100 mcg), tetracycline (30 mcg) and trimethoprim (5 mcg). Their zones of inhibition around the discs were measured and interpreted according to the manufacturer's recommendations.

The study revealed maximum sensitivity of E. coli isolates to norfloxacin and ciprofloxacin (98%) and gentamicin (88%). These three drugs have earlier been considered very effective against E. coli isolates from diarrheic calves and travelers in India (Patil et al., 1999, Villa et al., 2000). Maximum resistance was observed against tetracycline (100%) followed by ampicillin (86%), spectinomycin (74%) and cephalexin, cephadroxil and nitrofurazone (71%). E. coli isolates from bovines, camels and goats have shown high resistance to tetracycline and ampicillin (Wray et al., 1993, Singh et al., 1996, Cid et al., 1996). Two isolates of E. coli were resistant to all the 13 antibiotics used in this study which can be a matter of serious concern from public health point of view.

Antibiogram of EHEC isolates: Out of the 100 *E. coli* strains tested *in-vitro* for their sensitivity to various antimicrobial drugs, 22 belonged to non-O157 EHEC serotypes. The treatment of EHEC infection with antimicrobial

¹ Corresponding author

Table 1
Percent antibiotic sensitivity of isolates of enterohaemorrhagic and enterotoxigenic *E. coli*

| Antimicrobial drug (mcg/disc) | Number (%) of sensitive EHEC isolates (n=22) | Number (%) of sensitive ETEC isolates (n=37) |
|-------------------------------|---|---|
| Ciprofloxacin (5) | 22 (100) | 35 (94.5) |
| Norfloxacin (10) | 22 (100) | 35 (94.5) |
| Gentamicin (30) | 18 (81.8) | 31 (83.7) |
| Trimethoprim (5) | 16 (72.7) | 7 (18.9) |
| Chloramphenicol (30) | 16 (72.7) | 13 (35.1) |
| Streptomycin (10) | 14 (63.6) | 13 (35.1) |
| Nitrofurazone (100) | 08 (36.3) | 10 (27.0) |
| Ampicillin (10) | 04 (18.1) | 0 (0.0) |
| Cephalexin (30) | 04 (18.1) | 7 (18.9) |
| Cephadroxil (30) | 04 (18.1) | 7 (18.9) |
| Spectinomycin (100) | 02 (9.0) | 22 (59.4) |
| Tetracycline (30) | 00 (0.0) | 00 (0.0) |
| Polymyxin-B (300 units). | 08 (36.3) | 16 (43.2) |

have not been yet fully recommended. However, antimicrobial drugs are effective in reducing the severity of infection and risk of haemolytic uraemic syndrome in humans (Lane and Alexander, 1990, Takeda et al., 1998). Resistance of EHEC to antimicrobial agents has also been reported (Kim et al., 1994, Thomas et al., 1996). Sensitivity pattern of 22 isolates of EHEC (Table 1) revealed highest sensitivity to norfloxacin and ciprofloxacin (100% each). The 81.8% isolates were sensitive to gentamicin and 72.7% isolates to trimethoprim and chloramphenicol. Such high percentage of sensitivity of EHEC to a similar group of antimicrobials have earlier been reported (Ito et al., 1997, Sakata and Maruyama, 1998, Banerjee, 1999). The sensitivity of EHEC isolates to streptomycin, nitrofurazone, polymyxin-B, ampicillin, cepahalexin, cephadroxil and spectinomycin varied from 9% to 63.6% while none of the isolates was sensitive to tetracycline. Based on the results of this study, ciprofloxacin and norfloxacin may be the drugs of choice against EHEC isolates of non-O157 serotypes. Antibiogram of ETEC isolates: Among the 37 isolates of ETEC tested, 35 (94.5%) isolates were sensitive to norfloxacin and ciprofloxacin while 31 (83.7%) to gentamicin. Vila et al. (2000) reported a high sensitivity of ETEC isolates from travelers to ciprofloxacin and nalidixic acid. Patil et al. (1999) also showed sensitivity of E. coli

from diarrheic calves to norfloxacin (94.5%) and gentamicin (89.0%). Nitrofurazone was effective against 10 (27%) isolates while cephalexin, cephadroxil and trimethoprim were found least effective (Table 1). All the 37 isolates were resistant to tetracycline and ampicillin.

The present study reveals that isolates belonging ETEC and EHEC were highly sensitive to ciprofloxacin (94.5-100%), norfloxacin (94.5-100%) and gentamicin (81.8.-83.7%), but 100% resistant to tetracycline. These isolates showed moderate to high resistance to polymyxin-B, nitrofurazone, chloramphenicol, cephalexin, cephadroxil and ampicillin. Antimicrobial resistance is emerging and spreading among foodborne bacteria. These resistant bacteria when transmitted to human may be the cause of serious illnesses.

REFERENCES

Banerjee, R. (1999). Prevalence of verotoxic *Escherichia coli* in man, animals, foods and their public health significance. M.V.Sc. thesis, Deemed University, IVRI, Izatnagar.

Bauer, A.W., Kirly, W.M.M., Sherries, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path.* **45**: 493-496.

Cid, D., Blanco, M., Blanco, J.E., Quinterira, R.S.J.A., Fuente, R.De.La., Blanco, J. (1996). Serogroups, toxins and antibiotic resistance of *Escherichia coli* strains isolated from diarrheic goat kids in Spain. *Vety. Microbiol.* 53: 349-354.

Ito, T., Akino, E. and Hiramatsu, K. (1997). Evaluation of antibiotics used for enterohaemorrhagic *Escherichia* coli O157 enteritis - effects of various antibiotics on extracellular release of verotoxin. *Kansenshoguku* Zasshi J. Jpn. Assoc. Infect. Dis. 71: 130-137.

Kim, H.H., Samadpour, M., Grimm, L., Clausen, C.R., Besser, T.E., Baylor, M., Kobayashi, J.M., Neil, M.A., Schoenknecht, F.D. and Tarr, P.I. (1994). Characteristics of antibiotic-resistant *Escherichia coli* O157:H7 in Washington State, 1984-1991. *J. Infect. Dis.* 170:1606-1609.

Lane, W.M. and Alexander, K.C. (1990). Haemolytic uremic syndrome in children. *Postgraduate Med.* **88**: 135-140

Patil, R.M., Karpe, A.G. and Gujar, M.B. (1999). Studies on drug resistance and virulence of *Escherichia coli* from diarrheic calves. *Indian. J. Comp. Microbiol. Immunol. Infect. Dis.* **20**: 29-31.

Sakata, H. and Maruyama, S. (1998). Result of antibiotic therapy for children with *Escherichia coli* O26: H11 infection. *Kansenshogaku Zasshi*. **72**: 1-4.

- Singh, B.R., Kapoor, K.N., Kumar, A., Agarwal, R.K. and Bhilgaonkar, K.N. (1996). Prevalence of enteropathogens of zoonotic significance in meat, milk and their products. *J. Food Sci. Tech.* **33**: 251-254.
- Takeda, T., Yoshino, K., Uchida, H., Ikeda, N. and Tanismura, M. (1998). Early use of fosfomycin for Shiga toxin-producing *Escherichia coli* O157 infection reduces the risk of hemolytic uremic syndrome. *Int. J. Food Microbiol*, **66**: 103-110.
- Thomas, A., Cheasty, T., Frost, J.A., Chart, H., Smith, H.R. and Rowe, B. (1996). Verocytotoxin producing *Escherichia coli* particular serogroup O157 associated

- with human infection in England and Wales: 1992-1994. *Epidemiol. Infect.* **117**: 1-10.
- Vila, J., Vargas, M., Ruiz, J., Corachan, M., Teresa, J.D.A.M. and Gascon, J. (2000). Quinolone resistance in enterotoxigenic *Escherichia coli* causing diarrhea in travelers to India in comparison with other geographical areas. *Antimicrobiol. Agents Chemotherapy* 44: 1730-1733.
- Wray. C., McLaren, I.M. and Carroll, P.J. (1993). *Escherichia coli* isolated from farm animals in England and Wales between 1986 and 1991. *Vet. Rec.* 133: 439-442.