MINIMUM INHIBITORY CONCENTRATIONS OF Escherichia coli ISOLATED FROM CATTLE, PIGS AND CHICKENS IN INDONESIA

Sumadi, Istiyaningsih, Kamaluddin Zarkasie and Syamsul Bahri Siregar
Veterinary Drug Assay Laboratory
Gunungsindur, Bogor 16340 Indonesia

ABSTRACT

Minimum Inhibitory Concentration (MIC) of *Escherichia coli* isolated from cattle, pigs and chickens in Indonesia in 1986 was determined by using 6 kinds of antibiotics and chemotherapeutics; oxytetracycline hydrochloride (OTC), chloramphenicol (CP), dihydrostreptomycin (DSM), sulfa dimethoxine (SA), kanamycin (KM) and amino benzyl penicilline (ABPC). In this study 104, 107 and 108 strains of *E. coli* from cattle, pigs and chickens was used, respectively. Distribution curve of MIC for *E. coli* strains showed that most of all curve had 2 peaks, one was sensitive peak and the other was resistant one. Especially, two peaks can be devided clearly in OTC, DSM and SA, and resistant peaks in *E. coli* isolated from chickens were very high in the drugs. Moreover, the resistant limits of OTC, DSM and SA were 6.26, 6.26 and 400 μg (p) per ml, respectively.

INTRODUCTION

Since Moore et al. (1946) reported that a small amount of streptomycin added to the diet promoted growth of chickens, such many growth-promoting agents as antibiotics and synthetic chemotherapeutics have been used as feed additives to increase the production of animal protein from livestock and poultry. The prolonged use of these drugs of growth promotion, however, leads to a high incidence of enteric bacteria with drug resistance.

In Indonesia, several investigators performed the survey on the drug resistance (Budiharta and Waruju, 1985; Mudigdo and Peranginangin, 1982; Ronohardjo et al., 1985). Almost of them determined the drug resistance of several species of bacteria by using the disk method. However, in orde to carry out basic studies of drug resistance, this method seems to be insufficient. For example, distribution curves of MIC for bacteria to be tested is required to perform the survey on drug resistance.

This study was undertaken to elucidate MIC of *E. coli* isolated from cattle, pigs and chickens in Indonesia.

MATERIALS AND METHODS

Strains used

In 1986, the total of 104 *E. coli* strains from cattle, 107 strains from pigs and 108 strains from chickens in West Java and the suburb of Medan were isolated, respectively. In most cases, *E. coli* strains of cattle were isolated from rectal content, these strains were isolated from fresh fecal samples. A loopful of the fecal or rectal samples was cultured on deoxycnolate-hydrogen sulfide-lactose (DHL) agar plate (Eiken, Japan). These DHL agar plates were incubated overnight at 37°C. One colony resembling *E. coli* was picked up from each DHL plate and transfered to triple sugar iron agar slant (Eiken, Japan). They were than subjected to the IMViC system and identified as *E. coli*.

Drug

The following drugs were used; oxytetracycline hydrochloride (OTC), chloramphenicol (CP), dihydrostreptomycin (DSM), sulfadimethoxine (SA), kanamycin (KM) and amino-benzyl-penicillin (ABPC). DSM was dissolved in phosphate buffer (pH 8.0). The others were dissolved in sterile distilled water at concentration of 4 tp 16 ug (p) per ml. These stock solutions were further diluted with distilled water before use.

MIC

Heart infusion agar (HIA, Difco) plates containing serial two fold concentrations of each drug were prepared. For the test with SA, Mueller-Hinton agar (Difco) was used. Six-hour cultures of the test strains in heart infusion broth (HIB, Difco) were diluted about 100 times with sterile saline and inoculated into media with a multiple inoculator. Results were read after 18 hours of incubation at 37°C. MIC was defined as the lowest concentration of the drug that prevented growth, except for SA, for which the MIC was defined as the lowest concentration of drug inhibiting confluent growth of large colonies.

RESULTS

Most of all curves of MIC for *E. coli* strains had two distribution peaks.

In OTC (Fig. 1), sensitive peaks were observed at a concentration of 0.39 to 0.78 μg (p) per ml for strains tested. The peak for cattle isolates was the highest, followed by the peaks for pig and chicken isolates. The resistant peak observed at a concentration of 50 to 100 μg (p) per ml in chicken isolates was not sharp and there were two resistant peaks in pig isolates, one peak at a concentration of 50 to 100 μg (p) per ml and the other at over 100 μg (p) per ml. In cattle isolates, the low peak was observed at a concentration of 100 μg (p) per ml. The resistance limit of OTC was at a concentration of 6.26 μg (p) per ml.

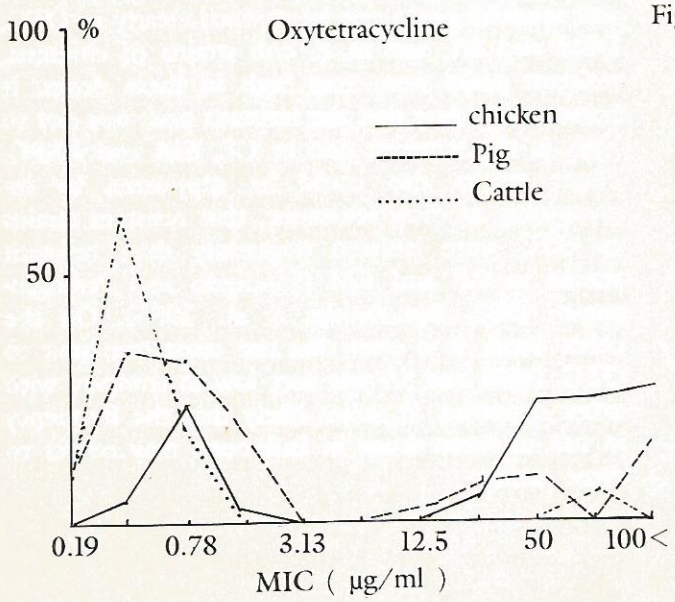

Fig. 1. Distribution curve of MIC for *Escherichia coli* strains isolated from animals

In DSM (Fig. 2), sensitive peaks were observed at a concentration of 0.78 to 1.56 μg (p) per ml for strains isolated from these animals. The peak for cattle isolates was the higest and the peak for chicken isolates was the lowest. On the other hand resistant peaks for chicken and pig isolates were observed irregularly. These isolates showed two peaks, respectively. Compared with these two peaks, the peak of chicken isolates was extremely higher than of pig and cattle isolates. The resistance limit of DSM was at a concentration of 6.20 μg (p) per ml.

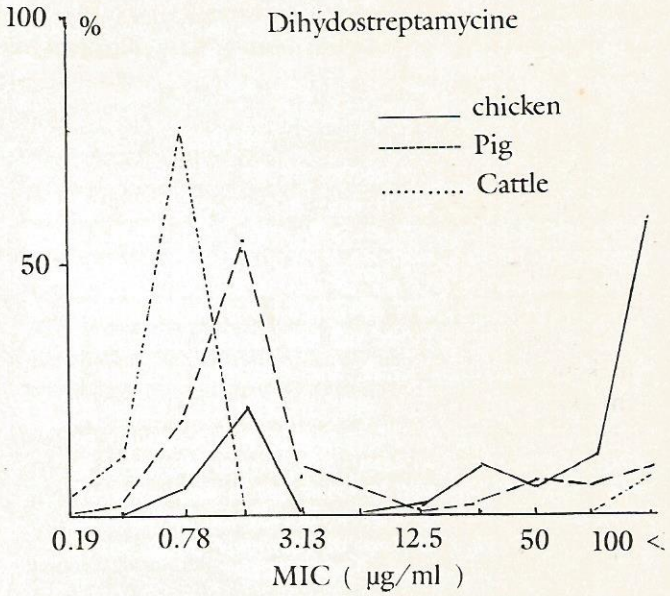

Fig. 2. Distribution curve of MIC for *Escherichia coli* strains isolated from animals

In CP, KM and ABPC (Fig. 3, 4 and 5), sensitive peaks were observed at concentration of 0.78 to 3. 13 μg (p) per ml for strains isolated from cattle, pigs and chickens. Among them, the peak for cattle strains was the highest. Resistant peaks were observed for strains isolated from pigs and chickens but both of peaks were not so high as the one shown in OTC.

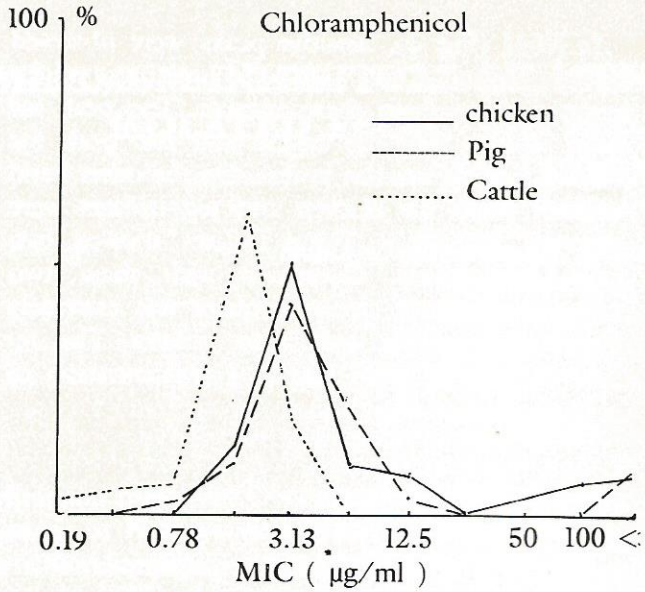

Fig. 3 Distribution curve of MIC for *Escherichia coli* strains isolated from animals

In SA (Fig. 6), sensitive peaks were observed at concentration of 12.5 and 50 μg (p) per ml for cattle and pigs isolates, respectively, but for chicken isolates, the sensitive peaks was observed irregularly. Resistant peaks were observed at concentration of over 1,600 μg (p) per ml for strains isolated from chickens and the peak for chicken isolates were remarkably high. The resistance limit of SA was at a concentration of 400 μg (p) per ml.

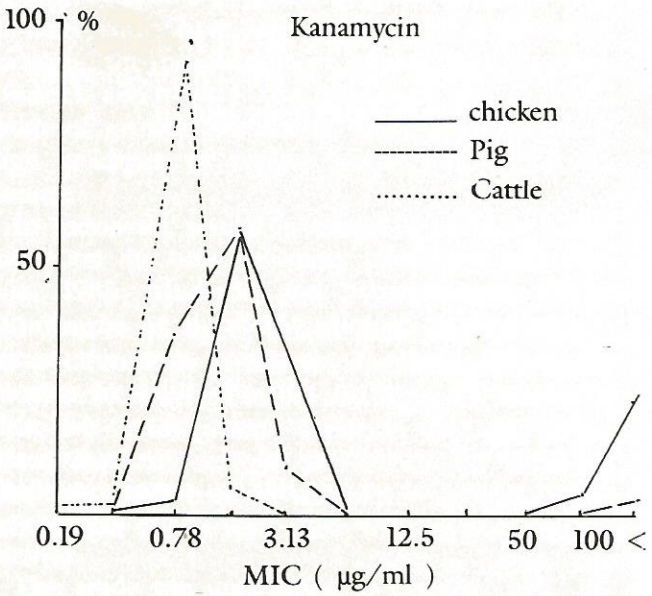

Fig. 4. Distribution curve of MIC for *Escherichia coli* strains isolated from animals

DISCUSSION

It is emphasized that this probably the first report about investigations of MICs in *E. coli* strains isolated from livestock and poultry in Indonesia. Author isolated *E. coli* strains from rectal contents or fresh feces as representatives of Enterobacteriaceae and investigate MICs.

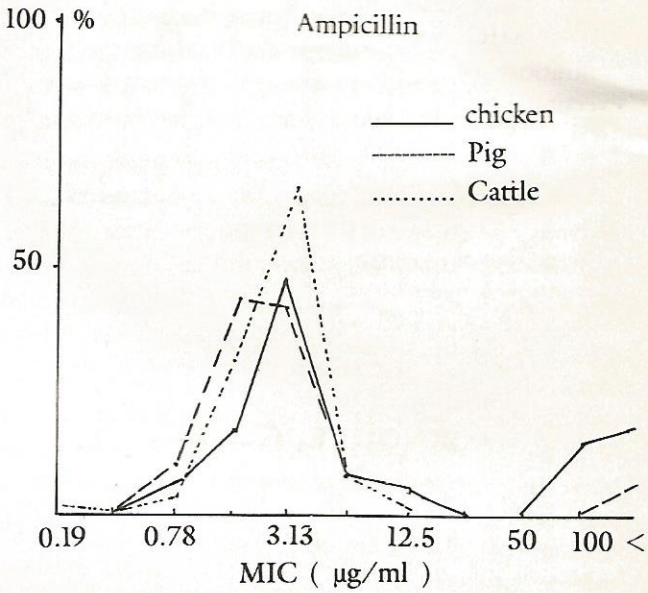

Fig. 5. Distribution curve of MIC for *Escherichia coli* strains isolated from animals

Judging from the results of MICs, the resistant and sensitive peaks can be devided clearly in OTC, DSM and SA, and in *E. coli* strains of chickens, resistant peaks were very high in these drugs. It became also clear that the resistant peak was not so high as the sensitive one in CP, KM and ABPC. These results also could be supported by investigating of drug resistance, that resistant strains to OTC were isolated frequently than resistant strains to CP and KM (Mudigdo and Peranginangin, 1982; Ronohardjo et al., 1985). On the other hand, it is clear that the resistance limit to OTC, DSM and SA was very low in compare with other countries. It seems likely that the frequency in use of these drugs in Indonesia may reflect to these results.

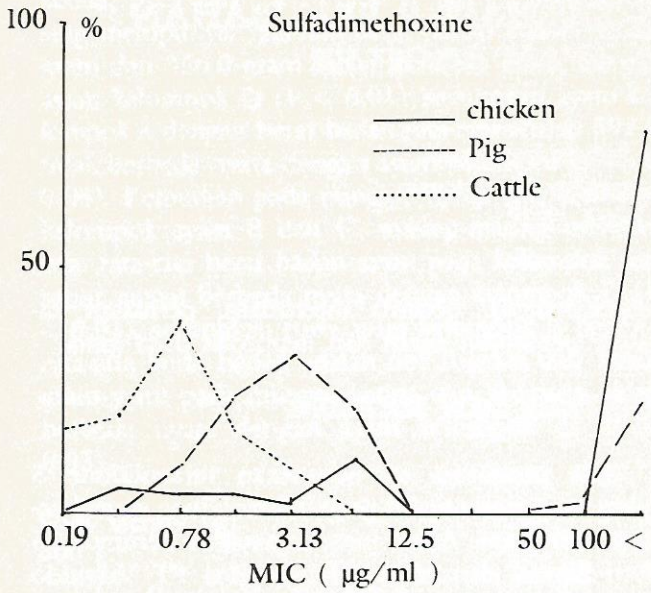

Fig. 6 .Distribution curve of MIC for *Escherichia coli* strains isolated from animals

ACKNOWLEDGEMENT

We are grateful to Dr. Masayuki Nakamura of National Veterinary Assay Laboratory, Japan for assistance in the preparation of this manuscript.

REFERENCES

Budiharta, S and B. Warudju. 1985. Mastitis di Daerah Istimewa Yogyakarta II. Isolasi bakteri penyebab dan resistensinya terhadap beberapa antibiotika. Hemerazoa. 72:58-68.

Harry, E.G. 1962. The ability of low concentrations of chemotherapeutic substances to induce resistance in *E. coli*. Research of Veterinary Science. 3 : 85-93.

Moore, P.R., A. Evenson, T.D. Lucky, E. Mckoy, C.A. Elveijem and E.B. Hart. 1946. Use of sulfasuxidine, streptothricin and streptomycin in nutritional studies with chick. Journal of Biological Chemistry. 165 : 437-441.

Mudigdo, R. and A. Peranginangin. 1982. Kejadian resistensi *Escherichia coli* terhadap tetracycline pada babi di daerah Medan dan sekitarnya. Annual report on animal disease investigation in Indonesia during the period of 1976-1981. p : 53-60.

Ronohardjo, P., S. Poernomo and L.H. Prasetyo. 1985. Pengujian in vitro imequyl terhadap berbagai kuman patogen gram negatif isolat lapang dari unggas. Penyakit Hewan. 17:301-304.

Smith, H.W. 1967. The effect of the use of antibacterial drugs, particularly as food additives, on the emergency of drug resistant strains of bacteria in animals. New Zealand Veterinary Journal. 15 : 153-166.

Smith, H.W. and W.E. Crabb. 1957. The effect of the continuous administration of diets containing low levels of tetracyclines on the incidence of drug-resistant *Bacterium coli* in the faeces of pigs and chickens : The sesitivity of the *Bacterium coli* to other chemotherapeutic agent. Veterinary Record. 69 : 24-30.