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ANTIMICROBIAL RESISTANCE OF *Escherichia coli* ISOLATES IN PIG FARM WORKERS, NORMAL AND DIARRHOEIC PIGS

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By

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Table		Page

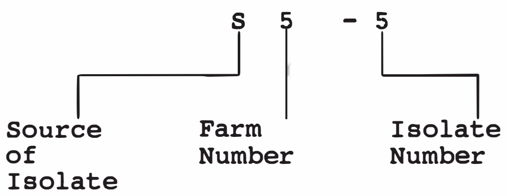
LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ap	ampicillin
C	chloramphenicol
Cf	cephalothin
CFU	colony forming unit
ccc	closed covalent circular
EDTA	ethylenediaminetetracetic acid
EMB	eosin methylene blue
Fr	furazolidone
Gm	gentamicin
kb	kilobase
K	kanamycin
MDa	megadalton
MPN	most probable number
N	neomycin
Na	nalidixic acid
P	probability
r^2	coefficient of correlation
S	streptomycin
Su	sulfisoxazole
SEM	standard error of the mean
SET	set buffer
Te	tetracycline
Tr	trimethoprim

Isolate Symbols



S - normal swine
B - diarrhoeic swine
SH - farm worker

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ANTIMICROBIAL RESISTANCE OF Escherichia coli ISOLATES IN PIG FARM WORKERS, NORMAL AND DIARRHOEIC PIGS

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Faculty : Veterinary Medicine and Animal Science

The enumeration of coliforms in faeces can be performed by both the spread plate method or Most Probable Number (MPN) techniques, although MPN total coliform counts are significantly higher ($P<0.05$). In solid agar media enumeration, MacConkey, Desoxycholate, Eosin-Methylene Blue and ENDO agars are suitable media for the enumeration of coliforms. The correlation coefficients of counts among media and technique is high. The mean colony forming unit (CFU) counts per gram for coliform in faeces varied from 2.6-6.4 X 10^7 and 1.8-2.7 X 10^8 for the pigs and farm workers tested respectively.

Random identification of 291 colonies on MacConkey agar on the basis of frequency of occurrence of numbers of lactose and non-lactose fermenters yielded 94.2% Escherichia coli (E. coli). The other genera isolated includes Enterobacter, Klebsiella, Edwarsiella, Citrobacter and Serratia. About 8.2% and 29.6% of the E. coli isolates were non-lactose fermenters on MacConkey agar and did not show the characteristic green metallic sheen on Eosin-Methylene Blue agar respectively.

Antimicrobial resistance to 11 antimicrobial agents of 274 *E. coli* isolates from pig farm workers, normal and diarrhoeic pigs were investigated. All isolates from the pig strains were resistant in contrast to 41.2% of farm workers isolates. The median antimicrobial resistance were 6.5, 3.8 and 0.8 for diarrhoeic pigs, normal pigs and farm workers respectively. Multiple resistance to at least 8 antibiotics were 27.9, 5.6 and 1.0% respectively in the same order. A total of 53 antibiotypes in 9 antibiogroups were detected. Antimicrobial resistances to antibiotics that were used subtherapeutically in feeds were associated with the highest resistances observed in normal pig isolates. Significant differences were observed in the mean antimicrobial resistance between normal and diarrhoeic pig isolates for ampicillin, gentamicin, kanamycin and neomycin ($P<0.05$) and trimethoprim ($P<0.01$). Individual farm preference for use of antibiotics in therapy were associated with higher frequencies of resistances observed for these drugs in pig isolates. The mean antimicrobial resistance were higher in larger farms ($P<0.01$) and in farms without a resident veterinarian ($P<0.05$).

All the randomly selected 67 *E. coli* isolates from the faeces of farm workers, normal and diarrhoeic pigs were able to transfer part or all of their antimicrobial resistance. The antimicrobial resistance transfer frequency ranged from 10^{-3} to 10^{-8} transconjugants per donor cell. Tetracycline, streptomycin and sulfisoxazole resistances were predominantly transferred. There was no significant difference ($P>0.05$) in the mean transfer frequencies of antibiotic resistance and the proportion of isolates that transferred antibiotic resistance for each antibiotic tested between pig and farm workers isolates.

The number of plasmids in 67 *E. coli* isolates from farm workers, normal and diarrhoeic isolates varied from 1-11 plasmids per isolate. In the isolates examined, 8 (11%) possessed more than 7 plasmids whilst 28 (40%) of the isolates possessed more than 5 plasmids. The median number of plasmids was 2.8, 4.0 and 4.9 plasmids in farm workers, normal pigs and diarrhoeic pigs respectively. The mean number of plasmids was significantly lower ($P<0.05$) in farm worker isolates (3.6 $\pm$ SEM 0.8) than in normal pig isolates (4.9 $\pm$ SEM 0.5) or in diarrhoeic pig isolates (5.2 $\pm$ 0.2) Multiple plasmid carriage was observed in 88.5%, 93.1% and 66.7% in normal pig, diarrhoeic pig and farm worker isolates respectively. There was no significant difference ($P>0.05$) observed in the number of plasmids harboured between isolates with increasing antibiotic resistance. The number of plasmids harboured in isolates was significantly higher in normal pig isolates ($P<0.05$) derived from farms without veterinary supervision than in farms with veterinary supervision.

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KERINTANGAN ANTIBIOTIK Echerichia coli PADA PEKERJA LADANG, TERNAKAN BABI SIHAT DAN CIRIT-BIRIT

Oleh

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Penghitungan koliform dalam najis boleh dijalankan dengan menggunakan teknik piring sebaran atau teknik bilangan paling mungkin (MPN), walaupun penghitungan jumlah koliform MPN adalah lebih tinggi ($P<0.05$). Media-media yang sesuai untuk penghitungan koliform melalui teknik piring sebaran termasuk media MacConkey, Deoksikolat, Esoin-Metilina biru dan ENDO. Koefisen korelasi hitungan di antara media-media tersebut adalah tinggi. Julat koliform dalam najis ternakan babi dan pekerja ladang babi adalah dalam lingkungan 2.6-6.4 X 10^7 dan 1.8-2.7 X 10^8 unit pembentuk koloni/gram.

Pengenalpastian rawak ke atas 291 buah koloni yang tumbesar pada media MacConkey berasaskan kepada keujudan koloni penapai laktos dan bukan penapai laktos menghasilkan peratusan Escherichia coli (E. coli) sebanyak 94.2%. Lain-lain genera yang diasingkan termasuk Enterobakter, Klebsiella, Edwardsiella, Citrobakter, dan Serratia.

Lebihkurang 8.2% dan 26.9% pencilan E. coli adalah penapai laktos atas agar MacConkey dan tidak menunjukkan kilauan hijau pada agar Eosin-Metilina biru masing-masing.

Kajian kerintangan antibiotik terhadap 11 jenis agen antimikrobial ke atas 274 pencilan E. coli daripada pekerja ladang babi, anak babi sihat dan anak babi yang mengalami cirit-birit telah dijalankan. Kesemua pencilan dari anak babi menunjukkan kerintangan tetapi hanya 41.2% pencilan dari pekerja ladang menunjukkan kerintangan. Median kerintangan antibiotik ialah 6.5, 3.8 dan 0.8 bagi pencilan dari babi bercirit-birit, babi sihat dan pekerja ladang babi setiap satu. Kerintangan berganda sekurang-kurangnya lapan antibiotik ialah 29.7, 5.6 dan 1.0% dalam aturan yang sama. Sejumlah 53 antibiotaip dalam 9 kumpulan kerintangan telah dikesan. Kerintangan antimikrob terhadap antibiotik yang digunakan secara subtherapeutik mengait dengan kerintangan yang tertinggi dalam pencilan dari babi sihat. Perbezaan kerintangan antibiotik yang ketara telah ditunjukkan di antara pencilan dari babi sihat dan cirit-birit untuk ampisilin, gentamicin, kanamicin dan neomicin ($P<0.05$) dan trimethoprim ($P<0.01$). Penggunaan antibiotik untuk rawatan ternakan cirit-birit adalah berkait dengan peratusan kerintangan yang lebih tinggi dalam isolat babi cirit-birit. Kajian ini juga menunjukkan kerintangan antimikrob ini adalah lebih tinggi bagi ladang yang besar ($P<0.01$) dan ladang yang tidak mempunyai perkhidmatan veterinar sepenuh masa ($P<0.05$).

Kesemua 67 pencilan E. coli yang dipilih secara rawak daripada pekerja ladang babi, babi sihat dan babi cirit-birit mempunyai kebolehan memindah kerintangan antibiotik secara bahagian atau sepenuhnya. Kekerapan pemindahan kerintangan adalah dalam lingkungan 10^{-3} hingga 10^{-8} transkonjugat per sel penderma.

Pemindahan kerintangan lebih banyak berlaku bagi tetrasiklin, streptomisin dan sulfisokazol. Kajian ini menunjukkan tiada ada perbezaan ketara dalam kadar purata pemindahan kerintangan antibiotik tidak kira sumber pencilan dan jenis antibiotik yang dikaji (P>0.05).

Bilangan plasmid pada 67 pencilan E. coli daripada pekerja ladang, babi sihat dan cirit-birit ialah di antara 1 hingga 11 plasmid setiap pencilan. Pada pencilan yang dikaji, 8 (11%) mempunyai sekurang-kurang 7 plasmid manakala 28 (40%) daripada jumlah pencilan mempunyai sekurang-kurang 5 plasmid. Media plasmid adalah 2.8, 4.0 dan 4.9 pada pencilan pekerja ladang, babi norma dan babi cirit-birit. Min plasmid lebih rendah (P<0.05) bagi pencilan pekerja ladang (3.6 ± SEM 0.8) daripada pencilan babi norma (4.9 ± SEM 0.5) atau pada babi bercirit-birit (5.2 ± 0.2). Plasmid berganda telah diperhatikan dalam 88.5%, 93.1% dan 66.7% jumlah pencilan daripada anak babi norma, anak babi cirt-birit dab pekerja ladang. Tidak ada korelasi antara peningkatan kerintangan antibiotik dengan jumlah plasmid dalam sesuatu pencilan (P>0.05). Bilangan plasmid adalah lebih tinggi (P<0.05) dalam pencilan anak babi norma di ladang-ladang yang tidak mempunyai perkhidmatan veterinar dibandingkan ladang yang mempunyai perkhidmatan veterinar.

CHAPTER 1

INTRODUCTION

It has been well documented that large amounts of drugs are used in food-producing animals (Mercer, 1975), either in feeds (Lehmann, 1972) or medications in the control, prevention and treatment of disease or promotion of growth (Mercer et al., 1978). Mercer et al. (1978) anticipated that within two decades, nearly all food bearing animals will be exposed to some form of medication.

The use of antibiotics in domestic animals particularly at subtherapeutic levels in animal feeds has raised concern in many countries for many years (Gersema and Helling, 1986). The wisdom of use had been questioned as early as in 1953 (Braude, 1978). The main contention is that indiscriminate use of antibiotics in animals would lead to increase in the population of antimicrobial resistant bacteria which would subsequently endanger human health.

Three pertinent issues need to be addressed. Firstly, do the use of antibiotics in the animal industry result in increase antibacterial resistance? Secondly, is it a human health risk? Thirdly does the risk outweigh the benefits?

Many studies have demonstrated that resistant bacteria developed following inclusions of subtherapeutic levels of antibiotics in animal feed (Smith and Crabb, 1957; Linton and Howe, 1975; Langlois et al., 1978a,b; Langlois et al., 1983). The spread of antimicrobial resistant organisms from farm animals to farm workers have been demonstrated (Levy et al., 1976; Lyons et al., 1980) through the food chain (McDonagh et al., 1958; Hobbs, 1961; O'Brien et al., 1982).

The basis of resistance is genetically determined and in most cases the genes responsible are located on plasmids. Plasmids are extrachromosomal pieces of deoxyribonucleic acids (DNA) which replicate independently of the chromosome of the host cell and under most circumstances are not essential for the growth and metabolism of the host cells. The resistance against antibiotics may be transferred predominantly by conjugation of one bacterium to another through sex pili. Other means of resistance transfer are by transduction, transformation or in rare cases by protoplast fusion (Broda, 1979).

O'Brien et al. (1982) reported that resistant plasmids may be extensively shared between animal and human bacteria and that geographic and temporal epidemiological studies tend to show that, in the United States, multi resistant strains of Salmonella appear to be spread among animals and humans through the food chain. In contrast (Gardner, 1978) observed no colonization of the human alimentary tract with resistance organisms found in animals fed with antibiotics in families in direct contact with these animals. The wide use of antimicrobial agents has led eventually to the emergence of genes encoding resistance to it (Broda, 1979; O'Brien et al., 1982). The spread of resistance genes on plasmids and the selective overgrowth of strains with the resistance genes have led to an uneven distribution of resistance observed in bacterial populations (Farrar, 1985).

There has been no comprehensive study on antimicrobial resistance of enteric pathogens in food producing animals in Malaysia. The published literature on antimicrobial resistance in domestic animals in Malaysia were based on very limited number of isolates (Chin, 1983), to the range of antibiotics tested (Chin, 1983; Koh and Kok, 1984; Bahaman and Bong, 1985) or limited only to clinical isolates (Joseph *et al*., 1978). Plasmid profile analysis and its related molecular genetic tools is also relatively new in Malaysia.

In this study, attention is directed towards differences in the antimicrobial resistance and plasmid profiles of *E. coli* strains isolated from normal untreated piglets and treated piglets receiving parenteral and oral antibiotics. Antimicrobial resistance of *E. coli* strains isolated from normal farm workers were also determined. The differences in antimicrobial resistance between pig and farm worker isolates, however cannot be compared directly as they may not be epidemiologically linked.

The objectives of this study are to:-

a) elucidate, compare and contrast the antimicrobial resistance patterns of *Escherichia coli* isolated from the faeces of pig farm workers, normal piglets and diarrhoeic piglets.

b) demonstrate the presence of resistance plasmids in E. coli isolated from the three experimental groups.

c) elucidate the role of plasmids in conjugal transfer of antimicrobial resistance in E. coli isolates from pig and farm workers.

d) compare the spread plate and multiple tube fermentation enumeration techniques of coliforms in fresh faeces.

CHAPTER 2

LITERATURE REVIEW

Antibiotics

Antibiotics are a group of complex organic chemicals which are produced by microorganisms during their growth and which in minute quantities have a detrimental effect on other organisms. They are therefore metabolites produced by bacteria and moulds. These antibiotics are effective against some bacteria, rickettsia, viruses, fungi and a few helminths. In this dissertation, the sulphonamides, a group of complex synthetic organic chemical compounds with chemotherapeutic activity, have been included as an 'antibiotic'.

Use of Antibiotics in Domestic Animals and Humans

Antibiotics were originally developed for medical and veterinary purposes to control specific pathogenic organisms, but in 1949, it was discovered that certain antibiotics could increase the rate of growth of young pigs and chicks when included in their diet in small amounts. The growth promoting properties of these compounds were at first thought to be due to the presence of vitamin B_{12} in the antibiotic preparations, but it was later shown that the antibiotic gave greater response than the pure vitamin (McDonald et al., 1978).

A wide range of antibiotics have been tested for growth promoting activity and amongst the antibiotics commonly used as feed additives in livestock production are chlortetracycline, oxytetracycline, procaine penicillin, oleandomycin, tylosin and various forms of bacitracin (Gobble, 1964). In addition streptomycin, tyrothricin, gramicidin, neomycin, erythromycin, virginiamycin and flavomycin have been used as feed additives (McDonald et al., 1978). In the United Kingdom, Braude (1978) reported that chlortetracycline, oxytetracycline, flavomycin, virginiamycin and zinc bacitracin were the major feed additives used. Joseph (1977) reported the use of chlortetracycline, oxytetracycline, procaine penicillin, streptomycin, sulphonamides, tylosin, neomycin and spiramycin in animal feeds in Malaysia.

In the United Kingdom, 168 tons of antibiotics were used in animal production in 1967 (Swann's Committee Report, 1969). In Malaysia, a survey of 8 major pharmaceutical suppliers in 1977 showed that 72.6 tons of antibiotics were used in animal feeds whilst 9.7 tons were used in the treatment of livestock diseases (MALAYSIA, Poison's Board, 1977).

Two recent surveys of drug use in swine practice in Malaysia (Salam and Khaw, 1987; Yogendran, 1987) showed that chlortetracycline and oxytetracycline are still the major antibiotics used as feed additives. The other antimicrobials used in descending order of frequency were furazolidone, virginamycin, zinc bacitracin and tylosin. Yogendran (1987) further reported the use of chloramphenicol, sulphonamides, tylosin, streptomycin, neomycin, kanamycin, pencillins including ampicillin and amoxycillin in the treatment of livestock diseases. Spectinomycin, kitasamycin, colistine, erythromycin and gentamicin were also used but a lower frequencies.

In Malaysia, commonly used antimicrobial agents in human therapy are penicillin, ampicillin, cloxacillin, streptomycin, polymixin B, erythromycin, chloramphenicol, tetracycline, gentamicin, triple sulphas, nitrofurantoin, kanamycin and cephalosporin (MALAYSIA, Institute of Medical Research, 1986).

Antibiotics in Animal Industry

Growth Promotion

Antibiotics are used as feed additives in order to increase growth rate, improve feed utilization and reduce mortality and morbidity from clinical and sub-clinical infections. Medication of feed, particularly in the prevention and control of disease where management, hygiene, housing are sub-optimal, has enabled herd and flocks to be kept in large numbers in concentrated areas. This unfortunately has led to misuse of medication (Salam and Khaw, 1987).

Several mechanisms of actions explaining the role antibiotics as growth promoters have been proposed. These include a) direct growth promotion effect, b) metabolic effect, c) nutrient sparring effect in which antibiotics may reduce the animals' dietary requirements, d) disease control effect, e) improvement of digestion or absorption of certain nutrients and f) improved feed and/or water intake (Steele, 1984). The growth promotant effect is manifested through the prevention of destruction of food protein in the gut, inhibition of toxin producing organisms, the prevention of gut thickening and thus facilitating absorption as well as inhibition of bacterial destruction of vitamins and the favouring of certain bacterial species which synthesise vitamins (Steele, 1984). The precise mechanism of action of antibiotics as growth promotants is not known (Keenan, 1971; Steele, 1984).

Therapeutic Applications

The effectiveness of antibiotic therapy depends on the presence of sufficient concentration of the antibiotic for an adequate duration at the site of infection to kill, inhibit, or otherwise damage the organism. The concentration of an antibiotic in a tissue is determined by a) dose and frequency of administration, b) route of administration, c) the rate and extent of absorption of the drug, d) its extent of distribution and rate of elimination, e) physiologic-anatomic barriers to drug penetration or physiologic concentration and f) plasma protein or tissue binding (Prescott and Baggot, 1988).

The effectiveness of treatment also depends on a) susceptibility of the organism in relation to tissue drug concentrations, b) the state of host defences, c) the nature and severity of infection, d) the removal of necrotic tissue, pus or foreign bodies and e) duration of therapy (Prescott and Baggot, 1988).