UNIVERSITI PUTRA MALAYSIA

THE PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA ISOLATED FROM MEAT

LAI KAI FATT

THE PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA ISOLATED FROM MEAT

LAI KAI FATT

FACULTY OF VETERINARY MEDICINE AND ANIMAL SCIENCE

UNIVERSITI PERTANIAN MALAYSIA

1985/86

THE PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA ISOLATED FROM MEAT

by

LAI KAI FATT

AN ANIMAL INDUSTRY PROJECT PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE AND ANIMAL SCIENCE, UNIVERSITI PERTANIAN MALAYSIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE (DVM)

UNIVERSITI PERTANIAN MALAYSIA

SERDANG, SELANGOR

APRIL 1986

ACKNOWLEDGEMENT

The author wishes to express his appreciation and thanks to his supervisor, Dr. Saleha Abd Aziz for her guidance and encouragement throughout the course of the study.

His gratitude is also forwarded to En. Shah Bazil for his assistance which made this study a success.

ABSTRACT

Swabs from 20 beef and 30 pork carcasses in Shah Alam abattoir indicated presence of various species of organisms. The contamination by *E. coli* was 20% and 25% in pork and in beef respectively. The most common isolated organism from pork carcasses was *Staphylococcus aureus* (70%) whereas from beef, it was *Streptococcus* species (70%).

Among the 61 strains of *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus* species isolated, 32 were found to be antibiotic resistant. Among them, 50% were monoresistant and 50% were resistant to 2 to 3 antibiotics, while only one isolate (a strain of *S.aureus*) was resistant to 4 antibiotics. None was found to be resistant to all the 6 antibiotics.

Resistant *E. coli* from pork was more common compared to beef. *E. coli* in pork was found to be resistant to ampicillin, kanamycin, streptomycin and tetracycline, while in beef this organism was only resistant to ampicilin. In pork, most of the organism were resistant to tetracycline and streptomycin.

Resistance pattern of staphylococci was similar in pork and beef. Resistance to erythromycin, methicillin, penicillin and streptomycin was observed both in beef and pork carcasses. However, resistance to tetracycline was seen in pork but not in beef. *Streptococcus* species showed little resistance in both beef and pork carcasses.

CONTENTS

LIST OF TABLES

INTRODUCTION

Antibiotic resistant organisms, in particular enteric bacteria, have arisen in animals following the widespread and often indiscriminate use of antibiotics for therapy, prophylaxis and growth promotion. The main method by which these antibiotic resistant organisms could potentially flow from these animals to man is by handling of food and ingestion of their products.

Microbial contamination of food animal products, meat, milk and eggs would not only result in their spoilage but also would cause public health hazard if pathogenic and antibiotic resistant bacteria were present. The contamination of these products could stem from any stages of their production.

Some works have been carried out in Malaysia to determine the quality of meat, milk and eggs, particularly on microbial load and the presence of pathogenic bacteria; however, investigation into the presence of antibiotic resistant organisms in these products are lacking.

Thus, the objectives of this paper are: firstly, to assess the microbial flora of meat in abattoir; secondly, to detect potentially public health hazardous pathogens; thirdly, to determine the sensitivity of common bacteria isolated from meat to antibiotics; and fourthly, to compare the patterns of sensitivity between pigs and cattle.

LITERATURE REVIEW

The flesh of healthy animals is essentially sterile,

perhaps containing low number of bacteria from time to time, but the lower gut and the exterior of the animals harbour enormous number which, without care may be transmitted to the surface of the carcass during slaughter (5). Determination of the number and type of organisms on the surface of meat is important from public health point of view, to judge the effectiveness of sanitation during processing and to estimate the quality including shelf-life of meat (6). While contamination may have no health risk, it is the spoilage deriving from possible multiplication and the presence of pathogenic organisms that are of importance in quality assurance. Majority of the 27 known genera of bacteria isolated from fresh meat are saprophytes (7, Appendix I) which include Acinetobacter, Aeromonas, Pseudomonads, Alcaligenes, Flavobacterium, Coryneforms and various Enterobacteriaceae. Several species such as Staphylococcus aureus, Micrococcus, Pseudomonads, yeast, molds and coliforms are considered dressing microflora. Salmonellae, Staphylococcus aureus, Yersinia enterocolitica, Clostridium perfringens, Clostridium botulinum are considered potential pathogens (11), thus their isolation in raw meat is a serious health problem causing foodborne illness. Apart from the isolation of potential pathogens in food, studies have shown that the indiscriminate use of antibiotics has resulted in the increase in prevalence of resistant strains of bacteria in meat, milk and faeces of animals. These antibiotic-resistant organisms, on gaining access to the human gut and upon colonisation, can transfer their resistance to the coliform of the gut and create a reservoir of enteropathogenecity (10). In a study on the effect of addition

of tetracycline to chicken feed upon human, it was found that within a week, virtually all the faecal enterobacteriae of the chicken were resistant to this antibiotic and after 5 months, about 30% of the faecal samples from the dwellers contained over 80% tetracycline-resistant bacteria, many of these were multi-resistant (4). Faecal Escherichia coli isolated from rectal swabs of normal pigs from farms in Johore and Selangor showed resistance of 84% to tetracycline, 72% to sulphonamides, 26% to neomucin, 13% to chloramphenicol and none to nalidixic acid (1). The study done on antibiotic resistance of genus Salmonellae in United States in different species showed multi-resistance in about 80% of the cultures with an even higher percentage in cultures from swine (3). Sojka (12, 13), on a retrospective study on salmonellae isolated from animals in England showed a progressive increase in resistant pattern from 35% in 1975 to 65% in 1978. There is strong evidence that handling of contaminated raw meat was the most important route by which antibiotic-resistant E. coli and other organisms could reach man, especially in countries where animals carry substantial reservoir of antibiotic-resistant E.coli (9). The mean by which resistant E. coli may reach man is via food-chain where some of these organisms may contaminate meat and poultry carcasses. The phenomenon of changes in sensitivity mediated by plasmid or R-factor transferable among different members of the Enterobacteriaceae and some other species has been observed in a number of epidemics in different countries of the world involving Salmonella typhimurium in Great Britain, S. wien in

France, S. typhi in Mexico, Vietnam, and India, and Shigella dysenteriae I in Guatemala (6). Thus, such resistant strains of pathogenic organisms pose problem in treating diseases which ensure. Among the food animals, calves, pigs and poultry were found to excrete antibiotic-resistant organisms in higher numbers compared to sheep and adult cattle, as these animals regularly receive antibiotics in their feed.

MATERIALS AND METHOD

At the Shah Alam abattoir, 20 beef and 30 pork carcasses were sampled. Samples from pigs were taken at random while all the slaughtered cattle were sampled during each visit because of their small number.

The carcasses were swabbed prior to chilling with cotton swabs moistened with Tryptose Soy Broth (Oxoid). These moistened swabs were rolled onto the obturator muscles several times. The swabs were placed in thermoflask packed with ice and transported to the Bacteriology laboratory in the Department of Veterinary Microbiology and Pathology. In the laboratory, they were immediately cultured onto Blood Agar (Oxoid) and MacConkey Agar (Oxoid). After 24 hours of incubation at 37 ^{o}C, the plates were read. Plates without growth were re-incubated for another 24 hours. Colonies on MacConkey agar were classified as lactose and non-lactose fermenters. Colonies with different morphological characteristics on the same agar were selected and Grm-stained to determine each of the different bacterial isolates, then sub-cultured onto blood agar to obtain a pure culture. Gram-negative

rods were also sub-cultured onto MacConkey agar to differentiate lactose from non-lactose fermenters. The pure cultures were subjected to a series of biochemical tests. The species of bacteria were identified using A Diagnostic Manual of Veterinary Clinical Bacteriology and MYcology (8). The Modified Kirby-Bauer Method (2, Appendix II) was used for the antibiotic sensitivity test. Six different antibiotics were used for Gram-positive organisms, namely, penicillin (10 mcg), methicillin (5 mcg), chloramphenicol (30 mcg), tetracycline (39 mcg), streptomycin (10 mcg), and erythromycin (15 mcg). For enteric organisms, ampicillin (19 mcg), chloramphenicol (30 mcg), tetracycline (30 mcg), streptomycin (10 mcg), neomycin (30 mcg) and kanamycin (30 mcg) were used.

RESULTS

The results of the survey at the Shah Alam abattoir were tabulated as follows.

TABLE 1 : Organism isolated from carcasses at Shah Alam abattoir

A) Gram-positive organism species	n=20 Beef carcasses	n=30 Pork carcasses
Staphylococcus aureus	4 (20%)	21 (70%)
Staphylococcus epidermidis	1 (5%)	9 (30%)
-Streptococcus spp	14 (70%)	9 (30%)
Micrococcus spp	1 (5%)	6 (20%)
Corynebacterium spp	3 (15%)	9 (30%)
Bacillus subtilis	8 (40%)	1 (3%)
B Gram-negative organisms species		
Escherichia coli	5 (25%)	6 (29%)
Klebsiella pnuemoniae	2 (10%)	0
Serratia spp	6 (30%)	0
Flavobacterium spp	0	1 (3%)
Alcaligenes spp	1 (5%)	0
Aeromonas spp	3 (15%)	1 (3%)
Citrobacter spp	3 (15%)	1 (3%)
Entrobacter spp	4 (20%)	0
Pseudomonas spp	4 (20%)	0
Neissieria spp	2 (10%)	2 (7%)

Table 2 : Number of antibiotic resistant strains isolated from beef and pork carcasses

Beef carcasses n = 20	Antibiotics								
	A*	C	E	K	M	N	P	S	T
E.coli (5)**	1	0	ND	0	ND	0	ND	0	0
K.pneumoniae (2)	2	0	ND	0	ND	0	ND	0	0
S. Aureus (4)	ND	0	1	ND	1	ND	1	1	0
Streptococcus spp (14)	ND	0	2	ND	1	ND	0	1	ND
Pork carcasses n = 30									
E.coli (6)	2	0	ND	1	ND	0	ND	3	5
S.aureus (21)	ND	0	2	ND	7	ND	2	7	10
Streptococcus spp (6)	0	0	1	ND	0	ND	0	4	ND

=A= ampicillin C= chloramphenicol E= erythromycin

K= kanamycin M= methicillin N= neomycin

P= penicillin S= streptomycin T= tetracycline

** = Number of isolates

ND = Not done

Table 3 : Antibiotic resistant pattern

Strains	Carcass source	Resistant pattern	
E. coli	Beef	A	1
K. pneumoniae	Beef	A	2
Streptococcus spp	Beef	ES	1
Streptococcus spp	Beef	EM	1
S. aureus	Beef	EMPS	1
E. coli	Pork	AS	1
E. coli	Pork	AST	1
E. coli	Pork	T	2
E. coli	Pork	TK	1
E. coli	Pork	TS	1
S. aureus	Pork	T	
S. aureus	Pork	TM	1
S. aureus	Pork	TP	1
S. aureus	Pork	TSM	2
S. aureus	Pork	SM	2
S. aureus	Pork	SE	1
S. aureus	Pork	SME	1
S. aureus	Pork	SPM	1
Streptococcus spp	Pork	E	1
Steptococcus spp	Pork	S	4
		Total	32

DISCUSSION

The total number of genera of bacteria isolated form swabbings of surfaces of meat carcasses in this study was 15 in beef and 11 in pork, as compared to 27 genera of bacteria isolated from fresh meat (Appendix I).. This is because the swabs were cultured aerobically and isolation techniques for bacteria of veterinary importance only were used. Anaerobes and lactobacilli for example were therefore excluded. The result indicated tha absence of salmonella in beef and pork in the abattoir at the time of survey. Enrichment procedure were suggested to be useful as isolation rate of salmonella were low even in a large sample of beef carcasses (13).

The most common bacteria identified from pork carcasses were the Gram-positive organisms, particularly the Staphylococcus SPECIES. Escherichia coli was the most frequent Gram-negative contaminant. It was suggested that excessive handling of pork carcasses would transfer staphylococci from human onto pork carcass, rather than contamination from skin, in which case more enteric organisms would be present (10). It is noteworthy that hair on pig skin is shaved manually rather than singed in the abattoir. In contrast, equal number of genera of Gram-negative and positive organisms were identified in beef carcasses. The Gram-positive cocci were again predominant, with Streptococcus species as the most frequent isolates. These findings do not indicate microbial load in beef and pork carcasses as enumeration of microbial count was not performed. The contamination by E. coli was low; 25% (5/20) in beef and 20%

(6/30) in pork carcasses.

The resistance patternon of E. coli and Klebsiella pneumoniae differed greatly between beef and pork carcasses. A greater number of resistance to antibiotic were found in pork than in beef (table 2). The findings could not be interpreted statistically as more values are required, from different abattoirs and perhaps from a series of isolation, to confirm with the observed higher rate of resistant strains. Various multi-resistant combination were found in pork than in cattle; of the 17 multi-resistant isolates only 3 were found in beef.

Resistance to tetracycline was seen in 5 of the 6 isoalted E. coli in pork but none in beef. High percentage of resistant strains to tetracycline were also reported in rectal swabs of normal pigs from Selangor and Johore farms (1). Similarly, resistant strains of porcine E. coli to streptomycin (3/6) was observed to have high percentage in contrast to that of bovine. None of the porcine and bovine E. coli isolates was found to be resistant to all the six antibiotics.

Neomycin, kanamycin and chloramphenicol resistant strains in porcine do exist as indicated in studies by Bahaman et al., (1) however, the small samples did not illustrate the extent of prevalence. Only 1 isolate from beef E. coli was observed to be resistant to ampicillin. The lack of resistant strains in cattle compared to the prevalence in pig clearly demonstrated the effect of widespread used of antibioics in pig industry; although studies have indicated that naturally occuring resistant strains do exist even before the introduction of antibiotics (4).

Similar pattern of resistance to antibiotics was observed

in Staphylococcus aureus in beef and pork carcasses as in E. coli. Nine of the 21 isolates of S. aureus showed resistance to 2 or 3 antibiotics, but none to all the six antibiotics (Table 3). In cattle, only 1 isolate of S. aureus was found to be resistant to 4 antibiotics, namely, penicillin, methicillin, streptomycin, and erythromycin. Resistance to tetracycline was observed in 10 of the 21 isolates (Table 2). Seven isolates were resistant to streptomycin and 7 isolates to methicillin. Methicillin was a new synthetic penicillin used in man, and rarely in animals. This would suggest a tranfer of human staphylococci to meat.

Streptococcus species isolated from pork and beef carcasses were not differentiated into faecal streptococci or non-faecal streptococci, but according to their haemolysis on Blood Agar, only α-haemolytic strains were isolated. Streptococcus species in pork and beef carcasses showed some resistance to the six antibiotics tested. None was observed to be resistant to penicillin.

CONCLUSION

The survey indicated that contamination with Escherichia coli was 25% (5/20) in beef and 20% (6/30) in pork. Salmonella was absent from meat swabs in the abattoir. Enrichment procedure may be useful for isolation of salmonella was suggested. Although sample size was samll, E. coli from pork was found to be more resistant to antibiotics as compared to those from beef. Multi-resistant was common in pork, however, none was observed

to resistant to all the six antibioics tested. Resistance to ampicillin, kanamycin and neomycin were less common in comparison to tetracycline and streptomycin. Resistance to ampicillin was observed in E. coli from beef. Resistance pattern for S. aureus was similar in pork and beef. Among the four species of bacteria, E. coli, K. pnuemonia, S. aureus and Streptococcus species, Streptococcus species from both pork and beef carcasses showed the least antibiotic resistant strains.

REFERENCES

1. Bahaman, A.R; Liman, B. The prevalence of antimicrobial resistant E. coli in pigs from Johore and Selangor. Malays. Appl Biol 14:19-23.

2. Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Turk, M. 1966. Anibiotic susceptibility testing by a standardized single disc method. Am J Clin Path 45:493.

3. Billi,O. B; Linde, K.S.; Merril, S. 1984. Antibiotic resistance of member of the genus Salmonella isolated from chicken, turkey, cattle and swine in the United States during Oc. 1981 through Sept 1982. Am J Vet Res AMVMS 45:245.

4. Broda,P. 1979. Plasmid. University of Edinburgh. W.H. Freeman & Co, Oxford & San Francisco. pp 101-124.

5. Butler, J.L.; Stuart, J.C.; Vauderzut, C.; Carpenter, Z.L.Smooth, G.C 1979. Attachment of microorganism to pork skin and surface of beef and lamb carcasses. J. Food Proct 42(5):401-406.

6. Eliot R.P.; Clark, P.S.; Lewis, K.H.; Lundbeck, H.; Olson, J.C.; Simonsen, B. 1978. Microorganisms in food I: Their significance and method of enumeration. 2nd Edition. University of Toronto Press. Toronto/Buffalo/London. pp 14,19,38.

7. James,M.J. 1978. Modern Food Microbiology. 2nd edition. Van Norstrant Reinhold CO. pp 77-78.

8. Jang, S.S.; Bibersiein, E.L.; Hirsh, D.c. 1978. A Diagnostic manual of Veterinary Clinical Bacteriologyand Mycology. University of California, Davis.

9. Linton,A.H. 1976. The antibiotic sensitivity testing of pathogens commonly found in vet practice. Vet. Rec. 99:370-371.

10. Robert, A.M.; Twed, T.; Bouton, B.K. 1979. Potential public health significance of non-escherichia coli coliform in food. J Food Potec 42:161-163.

11. Silliker, J.H.; Eliott, R.P.; Bard Parker,A.C.; Bryan, F.L.; Christian, J.H.B.; Clark, D.S.; Olson, J.C.Jr.; Robert, T.A. 1980. Microbial ecology of food: Food commodities international commission microbiological specification for food. Academic press. Vol 2 pp 340,344,345.

12. Sojka, W.J.; Hudson,E.B. 1976. A survey of drug resistance in salmonellae isolated from animals in England and Wales during 1972. Br Vet. J. pp 95-103, 121.

13. Sojka, W.J.; Wray, C. 1980. A survey of drug resistance in salmonellae isolated from animals in England and Wales from 1975 to 978. Br.Vet J. pp 136, 463-477.

Appendix I

27 known genera of bacteria found in fresh meat. (James, M.J. 1978)

Acinetobacter

Aeromonas

Alcaligenes

Arthrobacter (Coryneform)

Bacillus

Citrobacter

Clostridium

Corynebacterium

Enterobacter

Escherichia

Flavobacterium

Kurthia (Coryneform)

Lactobacillus

Bacteriodes

Leuconostoc

Microbacterium

Micrococcus

Moraxella

Niesseria

Pediococcus

Proteus

Pseudomonads

Salmonella

Serratia

Staphylococcus

Streptococcus

Streptomyces

The modified Kirby-Bauer Disc Technique

1. Five to six colonies from the sib-culture of the selected isolates were innoculated into 0.5 ml of sterile Tryptose soy broth (oxoid) and incubated at 37 degree celcius for 4 to 6 hours.
2. The concentration of the bacterial cells was standardized to that of one percent Barium Sulphate solution using sterile distilled water.
3. A sterile cotton swab was used to pick up the innoculum and it is then streaked onto a pre-heated Mueller-Hinton Agar plate (Oxoid). The streaking was repeated several times, each time by turning the plate at sixty degree, so as to ensure a complete lawn of bacterial growth.
4. Six types of antibiotic discs (oxoid) were place at equidistant from each other onto the innoculated Mueller-Hinto agar plate.
5. The plates were incubated aerobically at 37 degree celcius for 24 hours. The zones of inhibition were measure with a ruler and the sensitivity was interpreted as given in Appendix III.