

Antibacterial Resistance in the Muscles of Chicken, Pig and Beef

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ABSTRACT

Though antibiotic drugs are known to improve the health and welfare of food animals , there is parallel risk due to the development of resistant microorganisms in the body of target animals. Seven meat samples were procured from wet market in Old Town, Petaling Jaya, Malaysia and assessed for the presence of antibiotic residues. The samples chosen were chicken parts (skin, muscle and liver) , pig parts (liver, muscle and intestine) and beef muscle. The results indicated that chicken skin had high level of antibiotic residues which positively resisted the presence of gram positive, *Staphylococcus aureus*, *S. epidermidis* and *B. cereus* as known by the zone of inhibition. The beef muscle also held residue which resisted *S. aureus*. Chosen bacteria along with the extracts of chicken skin, pig intestine and beef muscle were observed to be resistant to tetracycline hydrochloride, ciprofloxacin hydrochloride monohydrate and their combinations when tested at a concentration of 1 percent

Keywords- antibiotic drugs, animal feeds, antibiotic residues, meat products,

I. INTRODUCTION

The poultry contributes one-fourth of the meat that are being produced worldwide. It plays a major role in human nutrition as a source of protein. Poultry units in the recent past are able to produce market ready broiler chickens in not more than 42 days.

Higher productivity is reasoned for the usage of antibiotics in feeds as therapeutic agents. Few of the common antibiotics used in the poultry are chlortetracycline, bacitracin, avoparcin, neomycin, oxytetracycline, tylosin, lincosamides, cephalosporins and virginiamycin.

The advantages of using antibiotics in poultry are that it helps to produce disease-free animals capable of growing fast and harboring low density of microbial pathogens. Antibiotics at a concentration of 50-100 mg kg⁻¹ in the feed act as growth promoters and suppressors of noxious microorganisms. Consequently inherent resistance develops against chosen bacteria which are often pathogens to human beings [1]. Typically, the elimination of the susceptible bacteria in poultry farming is initiated when a drug or an antibiotic is applied through feed notably at a dosage that exceeds a therapeutic index.

Certain microbes are able to withstand the above concentration. These bacteria will increase their numbers million fold. Such shift normally takes place after several days of continued administration of therapeutics. These resistant bacteria will eventually become dominant and its transmittance will occur due to genetically defined resistant characteristics to the next lineage of the

Strains as well as to other bacterial type by plasmid-mediated mutation routes [2].

The intransigence of bacteria towards the antibiotics is the competence of its populace to endure the aftermath of inhibitory pressure of antimicrobial agents [3]. Radical and pervasive usage of antibiotics will result in the development of a pool of resistant genes in the ambience. According to Randall *et al.* [4] the fluoroquinolones which being used in broiler chicken is known to increase the resistance of *Campylobacter*. Aarestrup *et al.* [5] studied the use of avilamycin in broiler feed which eventually developed resistance to *Enterococcus faecium*. Such resistant microbes may enter into human system consequent to incomplete cooking or wrong handling of the animal tissue [6].

The occurrence of resistance in bacteria is demonstrated by a proliferation of the microorganisms with a rate higher than that of the minimal inhibitory concentration (MIC). The latter is construed as the lowest concentration of antibiotic needed to hinder 90 percent of the colonies of a specific organism [7]. Therefore any gradual increment in the resistance of bacterial population to antibiotics is a matter of concern [8].

Resistance is often accomplished by enzymatic action. *Staphylococcus*, which produces penicillinase, has the ability to disintegrate the molecular structure of penicillin by hydrolytic cleavage, thereby developing deliberate resistance. The resistance is also due to the ability of the microbes to sustain in the latency of the antibiotic without a candid interaction.

Therefore it is felt necessary to investigate the occurrence of antibiotic residues and antibiotic-resistant bacterial strains in the meat of poultry, pork and beef being sold in the open market

II. MATERIALS AND METHODS

This work has the endorsement of the Research Ethics Committee of the Northumbria University and was designed so as to qualitatively evaluate the presence of any antibiotic residues in the edible parts of animal flesh procured from the market. In addition to the above a cursory test was made on the resistance of eight chosen bacteria (Gram+ive = *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus epidermidis*, *Bacillus cereus*, Gram-ive= *Escherichia coli*, *Proteus* sp., *Salmonella* sp. and *Pseudomonas* sp.) against two antibiotics (tetracycline hydrochloride and ciprofloxacin hydrochloride monohydrate) being popularly used in the husbandry of poultry and cattle.

Various parts of poultry (skin, liver, and muscle), pig (liver, muscle and intestine) and beef muscle were procured from the wet market in Old Town, Petaling Jaya, Malaysia.

Meat sample (5g each) was weighed, macerated in sterile mortar and pestle and emulsified with 10 mL phosphate-buffered saline (PBS), centrifuged at 5000 rpm for 10 min. and the supernatant was sterilized at a pressure of 15 psi for 15 minutes.

Parallely filter discs were placed in a clean petridish and sterilized. The sterilized extract was then transferred aseptically using a micropipette to the filter disc. Four types of filter discs (disc soaked with 80, 100, 120 and 140 μ L of the sterile extract) were thus prepared. Mueller-Hinton agar medium was prepared and plated in petridishes.

The 8 cultures grown in nutrient broths and incubated at 37°C for 24 h. were individually lawned on the sterilized Mueller-Hinton agar (MHA). Thus in each of the above plates one microbial culture was lawned. To that plates the filter disc incorporated with the tissue extract was placed. Each plate was demarked as four quadrates and each quadrate was placed with any one of the four filter discs of different concentration obtained from a particular tissue.

Thus there were 56 plates (7 tissue samples and 8 cultures) The plates were then inverted and incubated for 24 h at 37°C. On the expiry of the incubation, the plates were examined. The presence of antibiotic residues in the disc was inferred by the development of clear inhibition zone of a diameter of 0.5 mm or more.

In a next experiment the 8 microbes were individually cultured in nutrient agar broth. These cultures in broth were mixed with the sterile tissue

(chicken skin, pig intestine and beef muscle) extracts.

The contents were then plated in petri dishes in nutrient agar medium and incubated for 3 days. There were 72 petri plates (8 cultures, 3 tissue extracts, 3 antibiotics and its combination) Sterile filter discs were also prepared and were incorporated with 100 μ L of 1 percent aqueous solution of the two antibiotics.

These discs were then transferred to the plates containing the microbial culture and incubated for 24 h at 37 °C. The zone of inhibition was examined.

The compound diffused from the filter paper in to the agar and consequently no colonies could grow unless the microbes resist the antibiotics.

The pig parts showed any antibiotic resistance neither to the Gram +ive nor to the Gram -ive bacteria tested. The beef muscle showed antibiotic resistance against *S.aureus* and there was negative impact for other microbial species.

The intensity of the resistance depends on the bacteria's sensitivity to that particular antibiotic. The absence of growth around the disk was an indirect measure of the ability of that compound to inhibit that organisms.

III. RESULTS

The bacterial species employed in this experiment is given in Table 1. Out of the 8 bacteria tested 4 are Gram positive and the rest are Gram negative.

Table 2 indicates that the extract of the chicken skin had antibiotic residues inhibiting Gram positive bacteria: *S.aureus*, *S. epidermidis* and *B cereus* while the chicken muscle showed resistance to the gram positive bacteria *S. aureus* alone (Fig-1). The chicken liver did not show antibiotic resistance to both gram positive and negative bacteria.

The chicken skin extract when mixed with the bacterial cultures uniformly resisted the antibiotics: tetracycline hydrochloride, ciprofloxacin hydrochloride monohydrate and tetracycline hydrochloride + ciprofloxacin hydrochloride monohydrate (Table

Table 1. Bacterial strains used, their ATCC number and Gram Status

Bacterial strain	ATCC strain number	Gram status
Staphylococcus aureus	29213	Gram Positive
Staphylococcus epidermidis	12228	Gram Positive
Streptococcus pyogenes	19615	Gram Positive
Bacillus cereus	11778	Gram Positive
Escherichia coli	25922	Gram Negative
Proteus sp.	7002	Gram Negative
Salmonella sp.	55105	Gram Negative
Pseudomonas sp.	29423	Gram Negative

Table 2. Qualitative assessment of the resistance of meat extract to eight bacterial cultures

Bacteria	Vol. of extract (µL)	Chicken			Pig			Beef	
		Skin	Muscle	Liver	Muscle	Liver	Intestine	Muscle	
S. aureus	80								
	100								
	120								
	140								
S. pyogenes	80								
	100								
	120								
	140								
S. epidermidis	80								
	100								
	120								
	140								
B. cereus	80								
	100								
	120								
	140								
E. coli	80								
	100								
	120								
	140								
Proteus sp.	80								
	100								
	120								
	140								
Salmonella sp.	80								
	100								
	120								
	140								
Pseudomonas sp.	80								
	100								
	120								
	140								

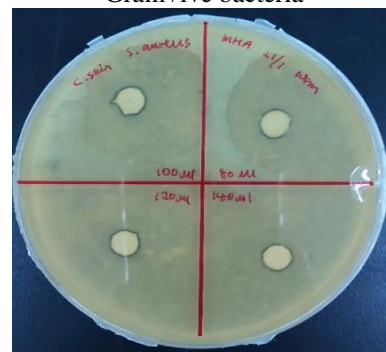
Extract expressing resistance

All the cultures when mixed with the extracts of pig intestine resisted tetracycline hydrochloride but could not resist ciprofloxacin hydrochloride monohydrate. When the antibiotics were mixed 1:1 ratio there was resistance. It clearly indicates that the effect is singularly caused by tetracycline hydrochloride even in the combination of antibiotics. In the case of beef extract the microbes resisted both tetracycline hydrochloride and ciprofloxacin hydrochloride. However they could not resist the combination of antibiotics.

Antibiotics	Meat		
	Chicken skin	Pig intestine	Beef muscle
Tetracycline hydrochloride			
Ciprofloxacin hydrochloride monohydrate			
Tetracycline hydrochloride + Ciprofloxacin hydrochloride monohydrate			

Table 3. The resistance pattern of the eight bacteria in combination with the extracts of chicken skin, pig intestine and beef muscle

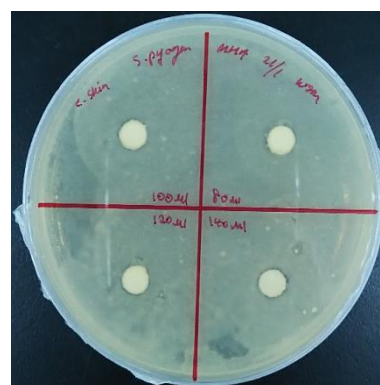
Fig1. Culture dishes showing inhibitory zone around chicken skin extract soaked discs against Gram+ive bacteria



S. aureus



S. epidermidis



S. pyogenes



B. cereus

IV. DISCUSSION

The microbes employed in this evaluation process have a far reaching pathological significance. *S. aureus* is known to create pimples, impetigo, cellulitis, folliculitis and abscesses. It may also be responsible for pneumonia, meningitis, osteomyelitis and sepsis. It secretes extra cellular polysaccharide which can coat any microbial films very easily. When a microbial film is caught within such coatings the nutrient transfer and antibiotic diffusion are hampered. *S. epidermidis* may bring skin rashes and are often resistant to popular antibiotics penicillin, amoxicillin and methicillin.

S. pyogenes is reported to cause rheumatic fever. *B. cereus* is responsible for causing nausea vomiting and diarrhea. Its spores survive if the food is cooked at or less than 100°C. The contamination normally takes a serious turn if the contaminated food is stored in a temperature range of 10- 50 °C for a long time as microbial proliferation takes place in that range of temp. *E. coli* is an indicator microbe for the fecal contamination of the food stuff. *Proteus* sp. is implicated as serious cause of infection in humans along with *Escherichia* sp. and *Klebsiella* sp. Contamination of this pathogen may occur due to improper handling of food products

Salmonella sp. may also be found in poultry, milk and eggs. Vegetables are also prone to be contaminated by this species

P. aeruginosa is known to develop resistance through mutation in chromosomally encoded genes or by horizontal gene transfer of antibiotic resistance determinants. Mutation driven antibiotic resistance in *P. aeruginosa* may be an irresistible factor for chronic infection

The increase in bacterial resistance to antibiotics and rising prevalence of bacterial strains with critical level of bacterial resistance in the food product is considered as a severe problem nowadays. The utilization of antibiotics brings about an upsurge in resistance to antibiotics not only in pathogenic bacterial strains but also in strains forming a part of the endogenous flora of humans and animals. Multi-resistant bacterial strains of animal origin may spread in to the human body by direct contacts or through food from animal sources.

These resistant strains colonize the human intestine and the genes coding resistance to antibiotics can be transferred to bacterial strains even that belong to natural micro flora [6]. The relation between the application of antibiotics and the dissemination of bacterial resistance from animal body to the human beings was described by Hummel *et al.* [9]. Leveyet *al.* [10] also confirmed that in chickens which was fed with tetracycline, there was transfer of tetracycline resistance genes

between chicken *E. coli* strains, from chicken to chicken and from chicken to man.

An ominous factor in this study is the demonstration of tetracycline and ciprofloxacin-resistant strains present in the chicken skin, pig intestine as well as beef muscle. Development of bacterial resistance to tetracycline and ciprofloxacin could have been caused due to the repeated administration of the two mentioned antibiotics.

The qualitative assessment of the presence of residues as studied showed that, chicken skin recorded the highest level of antibiotic residues as evident by the zone of inhibition in Gram positive bacteria, namely, *S. aureus*, *S. epidermidis* and *B. cereus*.

The evaluation of antibiotic resistance incurred a ban on subtherapeutic antibiotic usage in European Union and the potential for a ban in the United States as well as in many developed countries.

There is growing enthusiasm for identifying alternatives for antibiotics in feed and for the ratification of organic farming. Among the modern substitute feeds for animals, acidifiers are known to boost the growth of broiler chickens by lowering the pH of the feed. Thus inimical bacteria are prone to be fairly regulated. Probiotics, which are non-digestible substances beneficially influence the host by selectively stimulating growth and moderating the beneficial bacteria in the gut.

Probiotics often assist the productivity of animals through their resistance to diseases caused especially due to certain microbes such as *E. coli*, *Salmonella* spp., and *Clostridium perfringens*. The reduction of enteric disease in poultry and subsequent contamination of poultry products are some of their promising characteristics [11]. The most frequently used organisms in probiotic framework for poultry species are *Bacillus* sp., *Bifidobacterium* sp., *Lactobacillus* sp., *Streptococcus* sp. and *Enterococcus* sp. [12,13] which are found in large numbers in the gut of healthy animals and do not appear to affect them negatively. Present study qualitatively indicates the resistance of bacteria to some antibiotics in poultry feeds and underline the need of antibiotic policy implementation in veterinary medicine, including monitoring of bacterial strains with varying degree of innocuous phenotypes of resistance. Strict adherence of hygiene practice also plays an essential part in food safety and firmly regulating the transfer of resistant microbes from meat to humans. Poultry and beef meats need to be cooked assiduously to restrain human infection since due cooking procedures at high temperatures destroy the pathogenic bacteria. Indispensable hygienic measures coupled with cautious and more

diligent administration of drugs in animal husbandry are to be encouraged. It is strongly felt that the present qualitative evaluation needs to be validated through quantitative approach so as to provide a vivid picture on this issue .

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