African Scientist Vol. 17, No. 2 June 30, 2016
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2016030/17206

Antibiotic Resistance and Plasmid Profile of Bacterial Isolates from Pork Meat Retailed in Benin City

B. A. Omogbai* and L. N. Aghulor

Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

* Corresponding Author E-mail: barryomogbai@yahoo.com

(Received April 5, 2016; Accepted in revised form May 19, 2016)

ABSTRACT: The use of antibiotics in animal husbandry has helped in the reduction of animal diseases. However, health workers as well as enlightened consumers are becoming more concerned with the health implications such as resistance associated with the use of antibiotics since the same group of antibiotics are used in human medicine. Sixty (60) samples from pork meat, piggery environment and abbatoir effluent were screened for the presence of bacteria by selective and differential cultural procedures. Susceptibility of isolates to antibiotics and plasmid profiling were carried out using standard procedures. From the 60 samples analyzed, *E. coli* had the highest prevalence 49 (81.7 %) followed by *Staphylococcus aureus* 33 (55 %) and the least was *Klebsiella pneumoniae* 4 (6.7 %). In this study, it was observed that the most resisted drug was cefuroxime (93.3%) followed by ceftazidim (90 %) and the least was ciprofloxacin (13.3 %). The susceptibility pattern indicates that the bacterial isolates exhibited a varying level of resistance to two or more antimicrobial agents with maximum resistance to cefuroxime. Isolates were found to habour resistant plasmids of various sizes, but had susceptibility to antibiotics after plasmid curing. The detection of these organisms in pork meat may constitute a serious public health concern.The high rates of resistance found in this study can be explained by the wide use of antibiotics in Nigeria for pro-phylaxis and for treatment in animal farms.

Keywords: bacteria, isolates, pork , plasmid, resistance

Introduction

Pork is one of the perishable foods that potentially contain animal derived pathogenic bacteria. Thus, it constitutes a potential risk factor for spreading pathogens in its environment (Rieder *et al.*, 2012). Although pork is consumed for its nutritive value, the process of rearing animals as well as the process of preparing them for consumption is faced with many challenges. Notable among these challenges are: the use of antibiotics in animal husbandry, poor sanitary practices around slaughter houses, unhygienic methods of cooking as well as unhygienic retail systems amongst food handlers and meat sellers (Adesiji *et al*., 2011).

In the developing world, food-borne infection leads to the death of many children and the resulting diarrheal disease can have long-term effects on children's growth as well as on their physical and cognitive development (Yannick *et al.*, 2013). Food-borne diseases are diseases resulting from ingestion of bacteria and toxins produced by microorganisms present in food. Pork meat is a highly perishable food since it contains sufficient nutrient needed to support the growth of microorganisms. In most cases, during slaughter, dressing and cutting, microorganisms come

chiefly from the exterior of the animal and its intestinal tract. However, some also come from knives, cloths, air, and equipment in general. Retail cut could also result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrient and greater oxygen availability (Xavier *et al*., 2014).

The use of antibiotics in animal husbandry has helped in the reduction of animal diseases; however, health workers as well as enlightened consumers are becoming more concerned with the health implications associated with the use of antibiotics as it has been observed that the same group of antibiotics are used in human medicine. According to Nsofor and Iroegbu (2013), plasmids are a major mechanism for the spread of antibiotic resistant genes in bacterial populations. Conjugation occurs by F-plasmids that can transfer genes encoded for multiple resistance and mobilize other non-conjugative plasmids to host cells. *E. coli* has been reported to transfer the antibiotic resistance genes to enteric pathogenic and normal flora bacteria (Nsofor and Iroegbu, 2013).

The discovery of antibacterial agents in the first half of the 20th century radically changed the outcome of common human diseases. Many illnesses that were deadly before antibiotics became available are now readily treatable. The ability of bacteria to evolve mechanisms to resist attack by antimicrobials was recognized soon after the widespread deployment of the first antibiotics (DeWaal and Grooters, 2013). Cataloging foodborne illness outbreaks associated with antibiotic-resistant pathogens is a critical step in understanding the link between administering antibiotics to farm animals and human illness and, ultimately, preventing the problem (DeWaal and Grooters, 2013). Food contamination with antibiotic-resistant bacteria can also be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections (Adesiji *et al.*, 2011).

There is limited information on the antibiotic resistance of bacteria isolates from fresh and cooked pork meat retailed within a highly commercial city like Benin. Although contamination does not necessarily mean food-borne transmission, the possibility of these organisms being a food borne pathogen is worthy of investigation. This current study therefore, would be focused on assessing, isolating and characterizing microorganisms in cooked and fresh pork meat sold along the commercial streets of Benin. Antibiotic resistance plasmids would also be identified, with a view to highlighting the public health risk and implications of consuming such contaminated pork meat.

Materials and Methods

Sample collection

Sixty (60) samples of different portions of cooked and fresh pork meat as well as piggery environment and abbatoir effluent were obtained along the commercial street and various slaughter houses within Benin City. Of the 60 samples, twenty (20) each was obtained for the cooked and fresh pork meat while ten (10) samples each was obtained for piggery environment and abbatoir effluent. The samples were aseptically collected into clean sterile plastic containers and transferred to the laboratory in ice within 30 min for microbial analysis.

Sample preparation and culturing

Ten grams (10 g) of each meat sample was weighed and homogenized in 90 ml of sterile peptone water using a sterile mortar and pestle. Ten folds dilutions of the homogenates were made using sterile pipettes. From the 10-fold dilutions of the sample, 0.1 ml of the dilutions of the homogenate were plated on different media in duplicates, using pour plate and streaking method. The plates were then incubated at 37 °C for 24 - 48 h. Media employed for bacteria isolation include Nutrient agar (NA), MacConkey agar (MCA) and Selenite F broth. The media were prepared according to the manufacturer's specification and sterilized at 121 °C for 15 min prior to use.

Phenotypic identification of isolates

Bacterial isolates were characterized based on microscopic appearance and colonial morphology with Gram staining reactions, catalase and oxidase tests. Phenotypic profiling of both Gram-positive and Gram-negative organisms was undertaken using the API 50CHB and API 20E kit (BioMerieux, Marsielle, France) respectively. First a suspension of the test organism in 5 ml saline was made. From this suspension, sheep blood agar was inoculated for purity plate. All test chambers of the API kit were thereafter rehydrated by inoculation with a saline suspension of a pure culture of the bacterial strain subjected to identification. Incubation followed in a humidity chamber/tray for 18 to 24 h at 37 °C and the colour reactions were read. The results of the test reactions (plus the oxidase reaction which was done separately) were converted to a seven-digit code. Identity of the bacterium was then easily derived from the database with the relevant cumulative profile code book or software or fed into the

manufacturer's database via touch-tone telephone where the computer voice gives the genus and species identification of the test microorganism (TGHN, 2012).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was performed using disc diffusion according to Bauer *et al*. (1966). The following antibiotics (Oxoid, Basingstoke, UK) were tested: Gentamycin (10 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), Nitrofurantoin (30 µg), Augmentin (30 µg) Ampicillin (10 µg), Cefuroxime (30 µg) and Ceftazidine (30 µg). A suspension of the bacteria in 1ml sterile normal saline (0.98 %) was made to correspond to 0.5 McFarland standard. For *Salmonella, Staphylococcus* and *E. coli*, a sterile pipette was used to inoculate 100 µl of bacteria suspension onto Mueller–Hinton agar (Oxoid: CM337), For *Campylobacter*, the suspension was supplemented with 5 % (v/v) defibrinated sheep blood and 7 % w/w yeast extract. Each antibiotic disc was placed onto the agar with the aid of a sterile forceps and incubated at 37 °C for 24 h in air and microaerophilically for *Campylobacter*. The sensitivity plates were read and interpreted as recommended by NCCLS (2005) (now Clinical and Laboratory Standards Institute, CLSI).

Plasmid analysis

Plasmids were extracted using the alkaline SDS method (Kado and Liu, 1981). The extracted plasmids were separated by agarose gel electrophoresis for their profiling. Gel electrophoresis was carried out with 0.8% agarose in 1x TAE buffer (121 Tris-Base, 22.55 glacial acetic acid and 50 ml of 0.5M EDTA pH 8.0) on a horizontal gel apparatus at 100 V/cm2 for 3 h. The BAC-Tracker Super coiled DNA (Invitrogen) and 1KB plus DNA ladders (Invitrogen) were included for the estimation of plasmid sizes. The gel was stained with 5 µl of 10 mg/ml of ethidium bromide, visualized by UV trans-illuminator (Fisher Scientific, UK) and photographs were taken with Gel imager (Alpha Innotech Corporation, San Leandro, CA, USA).

Plasmid Curing

Nine (9) ml of freshly prepared nutrient broth was inoculated with a colony of various samples and incubated for 2-3 h for minimal growth of the microorganisms (turbidity), one (1) ml of Sodium Dodecyl Sulphate (SDS) curing agent was added to the mixtures obtained above, incubated for 24 h at 37°C and checked for growth. One (1) ml of the cured culture was inoculated into nine (9) ml of freshly prepared nutrient broth and incubated for 24 hours at 37°C. Pour plate method was carried out using the overnight broth culture to flood the nutrient agar plate. Then antibiotic discs were placed on the agar, incubated for 24 hours at 37 °C and results were read thereafter (Fortina and Silva, 1996).

Results

From the samples cultured (Table 1), *Escherichia. coli* had the highest prevalence (81.7 %) followed by *Staphylococcus* (55 %) while the least was *Klebsiella pneumoniae* (6.7 %). It was observed that *Bacillus* which had 30 % prevalence in the fresh pork was absent in cooked pork, while *Klebsiella* and *Salmonella* which were isolated from cooked pork samples were not present in fresh pork samples. However, the highest numbers of isolates were obtained from piggery environment and abattoir effluent samples.

From the antibiotic susceptibility result presented in Table 2, isolates that were resistant to more than two of the 8 tested antibiotics were regarded as multidrug resistant. It was observed that the most resisted drug was cefuroxime (93.3 %) followed by ceftazidim (90 %), ampicillin (88.3 %), augmentin (86.7 %), gentamicin (23.3 %), nitrofurantoin (15 %), ofloxacin (15 %) and then ciprofloxacin (13.3 %). The resistance pattern was such that almost all isolates irrespective of the source: whether from cooked pork, fresh pork, piggery environment or abattoir effluent reacted the same way to each of the drugs.

Table 1: Percentage (%) prevalence of bacteria in samples

	CPS n=20(%)	**FPS n=20(%)**	**PES n=10(%)**	**AES n=10(%)**	**Total N=60(%)**
Bacillus subtilis	Nil	6(30)	4(40)	1(10)	11(18.3)
E. coli	18(90)	12(60)	10(100)	9(90)	49(81.7)
Campylobacter jejuni	2(10)	1(5)	1(10)	3(30)	7(11.7)
Pseudomonas aeruginosa	5(25)	13(65)	8(80)	5(50)	21(35)
Proteus mirabilis	4(20)	10(50)	5(50)	6(60)	25(41.7)
Staphylococcus aureus	8(40)	6(30)	9(90)	10(100)	33(55)
Klebsiella pneumoniae	1(5)	Nil	2(20)	1(10)	4(6.7)
Salmonella typhi	3(15)	Nil	1(10)	1(10)	5(8.3)
Shigella flexneri	Nil	7(35)	6(60)	2(20)	13(21.7)
Enterobacter agglomerans	Nil	12(60)	3(30)	4(40)	19(31.7)

FPS = fresh pork samples , **CPS** = cooked pork samples, **PES** = piggery environment samples , **AES** = abattoir effluent samples

Table 2: Percentage resistance of isolates to test antibiotics from pork and its environment

Antibiotics	**FPB n =20 (%)**	**CPB n =20 (%)**	**PEB n =10 (%)**	**AEB n =10 (%)**	**Total n =60 (%)**
CPR	2(10)	1(5)	3(30)	2(20)	8(13.3)
OFL	2(10)	1(5)	3(30)	3(30)	9(15)
AUG	20(100)	12(60)	10(100)	10(100)	52(86.7)
NIT	2(10)	0	3(30)	4(40)	9(15)
AMP	19(95)	17(85)	9(90)	8(80)	53(88.3)
CAZ	20(100)	15(75)	90(90)	10(100)	54(90)
CRX	19(95)	17(85)	10(100)	10(100)	56(93.3)
GEN	4(20)	2(10)	4(40)	4(40)	14(23.3)

FPB = fresh pork bacteria , **CPB** = cooked pork bacteria, **PEB** = piggery environment bacteria , **AEB** = abattoir effluent bacteria

CPR= Ciprofkoxacin, OFL= Ofloxacin, AUG= Augmentin, NIT=Nitrofurantoin, AMP= Ampicillin, CAZ= Ceftazidin, CRX= Cefuroxime, GEN= Gentamycin

Plasmid analysis (Plate 1) showed that the isolates carried plasmids of various sizes which ranged between 1500 bp to 20000 bp. Of all the isolates, *Shigella flexneri* carried the highest number (5) of plasmids while *Campylobacter* carried the least number (1). The organisms *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus mirabilis* carried no plasmids (Table 3).

Table 4 shows the change in resistance pattern of 10 bacterial isolates after plasmid curing. The change is such that the organisms became susceptible to some of the drugs that they resisted before they were cured of plasmids. *Proteus mirabilis, Klebsiella pneumoniae* and *Staphylococcus aureus* after curing were only resistant to ampicillin. *Bacillus subtilis* on the other hand was totally sensitive to all the antibiotics after curing. The bacteria *Salmonella typhi, Escherichia coli* and *Campylobacter jejuni* however retained their multidrug resistance pattern without been affected by the curing (Table 4).

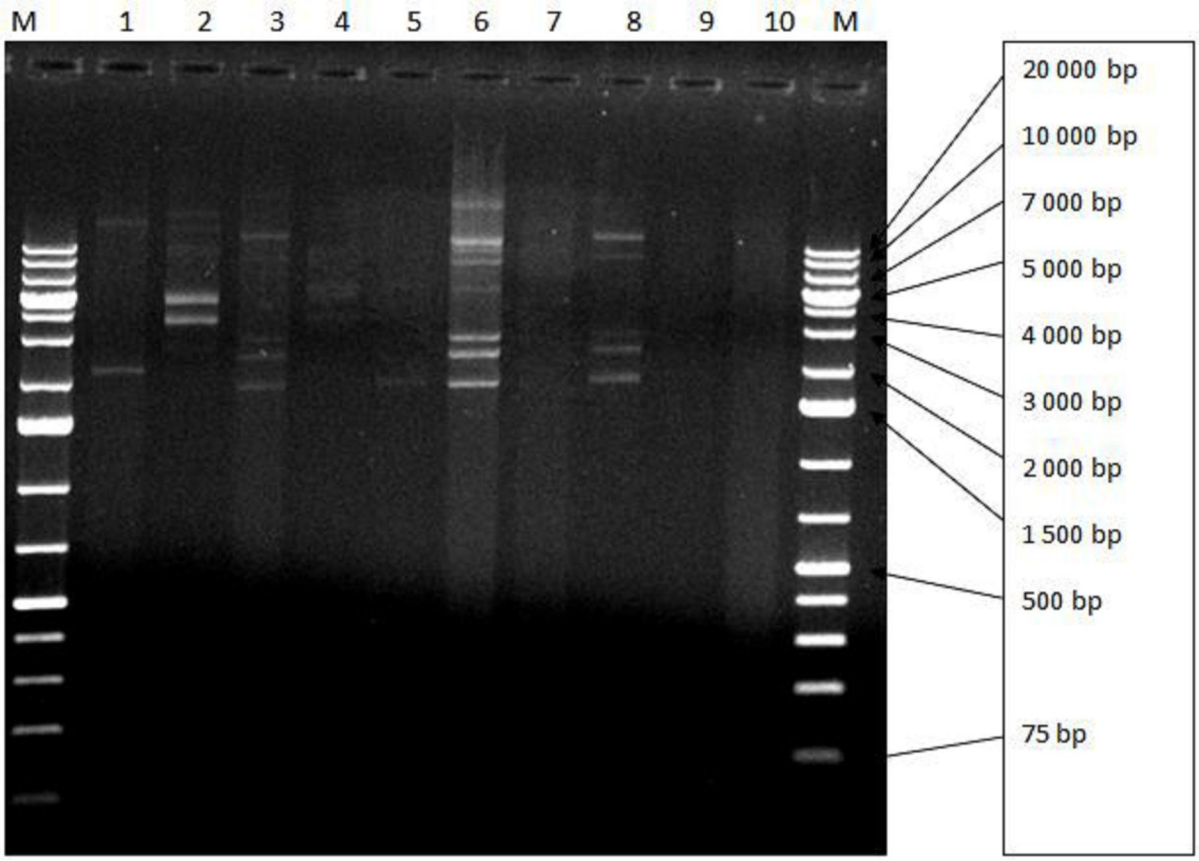

Plate 1: The plasmid profile of 10 multi drug resistant bacteria isolates from pork analysed with 0.8% agarose gel electrophoresis stained with ethidium bromide. M is DNA ladder (molecular marker). LANES : 1= *Escherichia coli*, 2= *Klebsiella pneumoniae*, 3= *Salmonella typhi*, 4= *Enterobacter agglomerans*, 5=*Campylobacter jejuni*, 6= *Shigella flexneri*, 7=*Bacillus subtilis*, 8 = *Pseudomonas aeruginosa*, 9=*Staphylococcus aureus*, 10= *Proteus mirabilis*

Table 3: Microbial isolates from pork and their plasmids

Lanes	Microbial isolate	Numer of plasmid borne	Plasmid size (bp)
1	*Escherichia coli*	2	2500, 20000
2	*Klebsiella pneumoniae*	2	4000, 5000
3	*Salmonella typhi*	3	2500, 3000, 10000
4	*Enterobacter agglomerans*	1	5000
5	*Campylobacter jejuni*	1	2500
6	*Shigella flexneri*	5	1500, 2000, 3000, 10000, >20000
7	*Bacillus subtilis*	0	Nil
8	*Pseudomonas aeruginosa*	3	2000, 2500, 10000
9	*Staphylococcus aureus*	0	Nil
10	*Proteus mirabilis*	0	Nil

Table 4: Antibiotic resistance profile of isolates before[(A)] and after[(B)] plasmid curing

Sample	Isolate	plasmid	CPR	OFL	AUG	NIT	AMP	CAZ	CRX	GEN	No. of drugs
A											
FPS1	*Pseudomonas aeruginosa*	+	S	S	R	S	R	R	R	R	5
FPS2	*Proteus mirabilis*	_	S	S	R	S	R	R	R	S	4
CPS1	*Staphylococcus aureus*	_	S	S	R	S	R	R	R	S	4
CPS3	*Salmonella typhi*	+	S	S	R	S	R	R	S	S	3
AES2	*Escherichia coli*	_	S	S	R	R	R	R	R	S	5
AES9	*Campylobacter jejuni*	+	S	S	R	S	R	R	R	S	4
PES3	*Klebsiella pneumoniae*	+	S	S	R	S	R	R	R	R	5
PES9	*Bacillus subtilis*	_	S	S	R	S	R	R	R	S	4
FPS3	*Shigella flexneri*	+	S	S	R	S	R	R	S	S	3
AES6	*Enterobacter agglomerans*	+	S	R	S	R	S	R	S	R	4
B											
FPS1	*Pseudomonas aeruginosa*	+	S	S	S	S	R	R	R	S	3
FPS2	*Proteus mirabilis*	_	S	S	S	S	R	S	S	S	1
CPS1	*Staphylococcus aureus*	_	S	S	S	S	R	S	S	S	1
CPS3	*Salmonella typhi*	+	S	S	R	S	R	R	S	S	3
AES2	*Escherichia coli*	_	S	S	R	R	R	R	R	S	5
AES9	*Campylobacter jejuni*	+	S	S	R	S	R	R	R	S	4
PES3	*Klebsiella pneumoniae*	+	S	S	S	S	R	S	S	S	1
PES9	*Bacillus subtilis*	_	S	S	S	S	S	S	S	S	0
FPS3	*Shigella flexneri*	+	S	S	R	S	R	R	S	S	3
AES6	*Enterobacter agglomerans*	+	S	R	S	R	S	R	S	R	4

FPS-fresh pork sample; CPS-cooked pork sample; AES-abattoir effluent sample; PES-piggery environment sample. CPR= Ciprofloxacin, OFL= Ofloxacin, AUG= Augmentin, NIT=Nitrofurantoin, AMP= Ampicillin, CAZ= Ceftazidin, CRX= Cefuroxime, GEN= Gentamycin

Discussion

The result of the isolates shows contamination with mostly enteric pathogens with the exception of *Staphylococus aureus* and *Bacillus subtilis.* The former is a normal flora of the animal skin while the later is a spore former which can be found in soil or as air-borne microflora from where it contaminates items including meat that is exposed such as pork meat vended along the street with little or no covering during sale to consumers. The high numbers of *E coli* and *Proteus* can be linked to the gut of the animal since many animals harbour these organisms in their gastro-intestinal tract as normal flora. The occurrence of *Pseudomonas, Campylobacter* and *Salmonella* is of public health importance as these food-borne pathogens can cause disease in humans when ingested. *Salmonella* and *Campylobacter* are known to cause life threatening gastrointestinal diseases- salmonellosis and campylobacteriosis. Although the result of this study are in concord with Yannick *et al*. (2013), *Shigella* spp was not isolated. Adesiji *et al*. (2011) observed that the most frequently isolated organism in chicken, beef and pork were *E. coli* (26%), *Arcobacter* spp. (19%), *S. aureus* (46%) and *Salmonella* spp 6 (2%).

The antibiotic resistance patterns of the isolates in this study differ with each drug, although a few were closely related. This may be attributed to the fact that each isolate reacted differently to a particular drug. Antibiotic resistance can be a result of horizontal gene transfer, and also of unlinked point mutations in the pathogen genome at a rate of about 1 in 10^8 per chromosomal replication. The antibiotic action against the pathogen can be seen as an environmental pressure. Those bacteria with a mutation that allow them to survive live to reproduce. They then pass this trait to their offspring, which leads to the evolution of a fully resistant colony (Kilonzo-Nthenge *et al*., 2008). Irrespective of the source of the sample, the isolates followed nearly the same resistance pattern. The result of this study is in consonance with Nsofor and Iroegbu (2013) who reported the resistance rate of 85% to ampicillin, 62.5% to nitrofurantoin, 56.3% to augmentin and 12.5% to gentamicin by microbial isolates from domestic animals.

The susceptibility pattern indicates that the bacterial isolates exhibited a varying level of resistance to two or more antimicrobial agents with maximum resistance to cefuroxime and highest susceptibility to ciprofloxacin. Multiple resistances were reported to be more common than resistance to single antibiotics. It was also reported that a high percentage of *E. coli* (88.2%) isolated from Swine faeces were resistant to one or more antibiotics (Nsofor and Iroegbu, 2012). In this study, all of the isolates were resistant to at least three or more antibiotics. However, the result shows that the fluoroquinolones: ciprofloxacin and ofloxacin are the most potent antibiotics among the drugs under study.

The high rates of resistance found in this study can be explained by the wide use of antibiotics in Nigeria for prophylaxis and for treatment in animal farms. It is well established that antibiotic pressure supports resistant strains and eliminates sensitive strains. The more antibiotics used, the more the elimination of the sensitive strains thereby, allowing resistant strains to dominate (Nsofor and Iroegbu, 2013). It is also true that resistant strains are outcompeted by sensitive strains when antibiotic pressure is removed from the environment. Thus, steps must be taken to control the overuse of antibiotics in Nigeria, as well as in other developing countries. The improper and unnecessary use of antimicrobial drugs in man also promotes development of resistant strains with R-plasmids. Nsofor and Iroegbu (2013) reported that both pathogenic and non-pathogenic strains of *E. coli* resistant to drugs may be transported from animals to humans via food. Such strains act as an important source for *in vivo* transmission of R-plasmids to drug sensitive strains in the animal intestine mainly through conjugation.

Antibiotics are used in food-producing animals to treat or prevent illnesses, for example, during the weaning period of young animals (Ahmadi *et al*., 2007). They may also be used for long periods at low levels to promote growth, increase feed efficiency, or compensate for unsanitary growing conditions on concentrated animal feeding operations (CAFOs) (Towner, 2000). Increased feed efficiency means animals require less feed per pound of weight gain, which translates into lower costs for producers. Many animal producers believe the use of antibiotics for growth promotion which also prevents disease (DeWaal and Grooters, 2013). Although a direct link between contamination of animal feeds and human disease has not been established, but the potential for transferring pathogens through feed exists and the findings of Nsofor and Iroegbu (2013) provide evidence to support studies that suggest the existence of a reservoir of antibiotic resistance genes. The use of foodstuffs that are free from pathogens will help reduce the risk of contamination of the animals (Dorea *et al*., 2010).

The isolates in this study had various plasmid sizes. However, plasmid sizes did not correlate with resistance pattern, thereby suggesting that not all antibiotic resistance genes are located in plasmids. Some of the genes conferring resistance could be located on bacterial chromosome. The findings of Nsofor and Iroegbu (2013) tend to agree with this result by stating that the plasmid DNA analysis of the strains shows that the size of the plasmids varied. Although, some strains were resistant to only four antibiotics, they had more than one plasmid while others containing 1 or 2 plasmids were resistant to a large number of antibiotics. However in their experiment, it was discovered that *E. coli* harboured resistance plasmids, which is in contrast to the findings of this study.

However, having carried out plasmid curing on isolates that were plasmid positive, the resistance pattern of the isolates changed. Some isolates had increased sensitivity (e.g. *Proteus, Staphylococcus and Campylobacter* which was previously resistant to four or five drugs became resistant to only ampicillin), while *Bacillus* which previously resisted four drugs became 100% susceptible. This confirms that plasmid carries resistance genes, but not in all cases. Many antibiotic resistant genes reside on transmissible plasmids facilitating their transfer. Exposure to an antibiotic naturally selects for the survival of the organisms with the genes for resistance. In this way, a gene for antibiotic resistance may readily spread through an ecosystem of bacteria. Antibiotic-resistant plasmids frequently contain genes conferring resistance to several different antibiotics.

Conclusion

Since fresh pork is not free of risk of contamination with bacteria, we can manage the level of risk with adequate consumer education. Proper storage and handling procedures must be used by the consumer to reduce the risk of food borne diseases. The need for microbial assessment of pork meats packaged for human consumption is therefore recommended to reduce possible contamination. Irrespective of the presence of these organisms in cooked pork meat analyzed, it is believed that cooking processes and good hygiene could greatly reduce the microbial load to harmless levels.

References

Adesiji MO, Adekanle MA, Jolayemi JB: Prevalence of *Arcobacter, Escherichia coli, Staphylococcus aureus and Salmomella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. S Leone J Biomed Res 3(1): 8-12. 2011.

Ahmad M, Ayremlou N, Sale HD: The effect of heat stress on the antibacterial resistance and plasmid profile in Escherichia coli isolates. Pak J Biol Sci 10: 4261-4265. 2007.

Bauer AW, Kirby MMW, Sherris JC, Turck M: Antibiotics susceptibility testing by standardize single disk method. Amer J Clin Pathol 36:493-496.1996.

Cereser A, Capelli G, Favretti M, Marchesan D, Marchesan R, Marcat M, Rossettok K, Furlan F: Prevalence of foodborne pathogens in rural pigs and in derived cold pork meats-preliminary report. Safe Pork 2007- Verona (Italy).110-112. 2007.

Clinical and Laboratory Standard Institute (CLSI): Performance standards for antimicrobial susceptibility testing; sixteen international supplements. CLSI document M100 S16. 26(3); M7-A7, 26(2); M2-A9, 26(1). Wayne PA. USA. 183p. 2006.

Davies RP: Intensive swine production and pork safety. Foodborne Pathogens Dis 8(2): 189-201. 2011.

DeWaal SC, Grooters VS : Antibiotic resistance in foodborne pathogens. Centre for Science in the Public Interest 1-13. 2013.

Dorea FC, Cole DJ, Hofacre C, Zamperini K, Mathis D, Doyle M P, Lee MD, Maurer JJ: Effect of *Salmonella* vaccination of breeder chickens on contamination of broiler chicken carcasses in integrated poultry operations. Appl Environ Microbiol 76:7820–7825. 2010.

Fortina MG, Silva M: Mappings of two plasmids from Lactobacillus helveticus ILC54, Plasmid curing and preliminary studies on their involvement in lactose metabolism and peptidase activity. Biotechnol Lett 18: 1003-1006. 1996.

Gebreyes AW, Bahnson BP, Funk AJ, McKean J, Patchanee P: Seroprevalence of *Trichinella, Toxoplasma* and *Salmonella* in antimicrobial-free and conventional swine production systems. Foodborne Pathogens Dis 5(2): 199-203. 2008.

Kado CI, Liu ST: Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 145: 1365-1373. 1981.

Kilonzo-Nthenge SN, Nahashon F, Chen H, Adefope N: Prevalence and Antimicrobial Resistance of Pathogenic Bacteria in Chicken and Guinea Fowl. Poultry Sci 87:1841-1848. 2008.

Lertworapreecha M, Sutthimusik S, Tontikapong K: Antimicrobial resistance in *Salmonella enterica* isolated from pork, chicken and vegetables in Southern Thailand. Jundishapur J Microbiol 6(1): 36-41. 2013.

Lopez-Campos G, Martinez-Suarez VJ, Aguado-Urda M, Alonso LV: Microarray detection and characterization of bacterial foodborne pathogens. Springfiels Briefs Food Health Nutr (11): 13-32. 2012.

McDonald RR, Antonishyn NA, Hansen T, Snook LA, Nagle E, Mulvey MR: Development of a triplex real-time PCR assay for detection of Panton-Valentine leukocidin toxin genes in clinical isolates of methicillin-resistant Staphylococcus aureus. J Clin Microbiol 43:6147-6149. 2005.

McMullen ML: Intervention strategies to improve the safety of pork. Adv Pork Prod 11: 165-173. 2000.

Moore T, Globa L, Barbaree J, Vodyanoy V, Sorokulova I: Antagonistic activity of *Bacillus* bacteria against food-borne pathogens. J Probiotics Health 1(3): 1-6. 2013.

Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schaffer AA: Database indexing for production MegaBLAST searches. Bioinformatics 24:1757-64. 2008.

Nsofor CA, Iroegbu CU: Plasmid profile of antibiotic resistant *Escherichia coli* isolated from domestic animals in South-East Nigeria. J Cell Animal Biol 7(9): 109-115. 2013.

Rieder G, Krisch L, Fischer H, Kaufmann M, Maringer A, Wessler S: *Carnobacterium divergens* – a dominating bacterium of pork meat juice. FEMS Microbiol Letters 1-9. 2012.

TGHN (The Global Health Network): Microbiology standard operating procedure: Bacterial identification using Biomerieux API kits, Biomerieux France, 21p. 2012.

Towner KJ: Resistance to antimicrobial agents. Antimicrobial Chemotherapy, 4th Edition, Oxford University Press Inc., New York. 2000.

Wieczorek RK, Szewczyk JO: Prevalence, antimicrobial resistance and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. Veterinarni Medicina 57(6): 293-299. 2012.

Wikler MA: Clinical, Institute LS. Performance standards for antimicrobial disk susceptibility tests: approved standard. Clinical and Laboratory Standards Institute. 2006.

Xavier C, Gonzales UB, Paula V, Estevinho L, Cadavez V: Meta-analysis of the incidence of foodborne pathogens in Portuguese meats and their product. Food Res Int 55: 311-323. 2014.

Yannick N, Niba R, Akwah E: Assessment of bacteriological quality of cooked pork meat sold along the commercial streets of Nkwen through Bambili Metropolis, Cameroon. Afr J Food Sci 7(12): 441-445. 2013.