International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

In Vitro Analysis of Antimicrobial Susceptibility Profile of Microorganisms Isolated from Pork Sold In Uyo, Nigeria

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Abstract— Background*: Isolation of microorganisms from roasted pork in Uyo have been conducted and reported by researchers, but information on the antimicrobial susceptibility profile of microorganisms isolated from this meat product is rare.*
Objectives: *The study was therefore conducted on in vitro analysis of antimicrobial susceptibility profile of microorganisms isolated from roasted pork sold in Uyo, Nigeria.*
Materials and Methods*: The samples were carefully collected from sales points and transported to the Microbiology laboratory, Department of Microbiology, University of Uyo for analysis. Samples were cultured on suitable microbiological media for bacteria and fungi using standard microbiological techniques. Each isolate was properly labeled with a code. The in vitro susceptibility testing of bacterial and fungal isolates was performed by means of Kirby-Bauer agar -disc diffusion method.*
Results*: A total of 31isolates comprising 26 bacterial and 5 fungal isolates were obtained in the study. The isolates and their frequency of occurrences were; Escherichia coli (29.1%), Staphylococcus spp (19.4%), Salmonella spp (16.1%), Enterobacter spp (12.9%), and Vibrio species (6.5%), Penicillium spp (9.1%), Aspergillus spp (6.5%). The susceptibility profile results revealed that many bacterial isolates obtained in the study expressed multi-drug resistance pattern. It was observed that E coli-EA1 expressed resistance to Streptomycin and Ceftazidime with lower ZI of 9 ± 0.9mm and NZ respectively but highly sensitive to Ciprofloxacin with wider zone of inhibition (ZI) of 37 ± 0.8mm. Staphylococcus aureus-EI1 was resistance to Ceftazidime and Streptomycin with ZI of 11±1.0mm and 10±1.8mm respectively. Ciprofloxacin and Gentamycin antibiotics were highly potent against majority of these bacterial isolates from pork. Aspergillus ssp-BD1 was observed with a wider Z.I of 31±1.3mm to Nystatin and 32 ± 0.8mm to Voriconazole respectively. Penicillium spp-IA3 was resistant to Fluconazole.*
Conclusion: *Some isolates from roasted pork expressed multi- drug resistance pattern but higher sensitivity was observed on Ciprofloxacin and Gentamycin antibiotics. Thus, thorough susceptibility testing is necessary before anti microbial prescription and administration to patients suffering from infections caused by these isolates.*

Keywords— *Pork, isolates, antimicrobial, resistance, infections*.

I. INTRODUCTION

The ability to manage and treat infectious diseases was greatly improved when various antimicrobial agents were discovered[1]. But the abuse of these antimicrobial agents in human therapy, farm animals and even for fish in aquaculture resulted in the spread of multiple drug resistant microorganisms in several communities[2-4]. However, since the beginning of the era of antimicrobial resistance, there is a good number of treatment failures as many strains of microorganisms are no longer sensitive to the drugs[5]. Thus, infection involving microorganisms poses a very serious public health problem all over the world especially in developing countries with limited resources. The condition has become global and most serious public health issues that increase in magnitude and speed every day [6,7,8]. Drug-resistant microorganisms are a growing danger to the global society. They endanger people in prosperous societies to poor nations with much severity with many infections, one of which is food borne infection.

Food - borne illnesses are infections with high mortality and morbidity especially in developed and developing countries [9,10]. According to World Health Organization, food - borne diseases encompass a wide spectrum of illness and are growing public health problem worldwide[11]. They are caused by pathogenic organisms of diverse genera when present in food and are ingested by humans. This results in gastrointestinal infections leading to irritation of the tracts and other related clinical symptoms. One source of food borne infection can possibly be pork that harbors microbial contaminants[12]. Pork is usually sold at joints and snack bars as ready- to - eat or take away foods. The business is on the increase and pork joints are gaining popularity among Nigerians, the pork sale points are located on some busy streets and roads. Many people in Nigeria especially in the South-Eastern part of the country patronize the sellers [12]. However, some of the food centers are poorly kept and are infested with flies, cockroaches, rats and other pests. These vectors transfer contaminants from contaminated feces to humans through the fecal-oral route[12,13]. Moreover, many researchers reported that pig harbors diverse groups of microorganisms [14,15]. These microorganisms could be transferred to pork, if not properly processed. The pork could also have cross-contamination effect if not properly handled and well packaged. These can constitute health hazard to consumers as it could cause food borne[14,15,16].

However, increasing prevalence of antimicrobial resistance is of global concern as they make the treatment of infectious diseases difficult. The determination of resistance profile of a given bacterium is very necessary. Given the danger as well as the complications arising from drugs- resistant organisms, resistant profile will therefore be useful in formulating rational antibiotic policy, treatment and management of patients with

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infections caused by microorganisms. Many researchers reported the isolation of microorganisms in roasted pork in Uyo and Nigeria as a whole[12, 16]. But information on their antimicrobial susceptibility profile of microorganisms isolated from this meat product is rare. The research sought to provide baseline information on the presence of antimicrobial resistant microorganisms in pork sold in Uyo. The research study therefore was conducted on *in vitro* analysis of antimicrobial susceptibility profile of microorganisms isolated from roasted pork sold in Uyo Metropolis. Akwa IIbom State, Nigeria.

II. Materials and Methods

Study Area: The study was conducted in Uyo metropolis from May, 2018 to July, 2018.. Uyo is the capital city of Akwa Ibom State. It is located between Latitudes $5^0$02'37 North and Longitudes $7^0$5'6" East with a fast growing population. The official population is estimated at 429,900 as at 2016[17].

Collection of samples

The pork samples used for this research work were obtained from pork selling points in major roads in urban area of Uyo, Akwa ibom State, Nigeria. The samples were collected aseptically, wrapped in a sterile aluminium foil and put in sterile containers. The samples were transported to the Microbiology laboratory, Department of Microbiology, University of Uyo, Akwa Ibom State, Nigeria for analysis using standard technique.

Processing of samples

The pork samples collected from each joint were carefully and aseptically processed in the Laboratory using methods recommended by [12,18]. Ten (10) grams of each sample was weighed out, and homogenized into 90ml of sterile distilled deionized water and vigorously shaken to dislodge adhered microorganisms. One (1) ml of the diluents was aseptically plated on the media in Petri dishes containing the following: prepared molten agar of Nutrient Agar (Oxoid, USA), MacConkey Agar (Oxoid, USA), Thiosulphate citrate bile-salt agar (Oxoid, USA), Eosin Methylene Blue (Oxoid, USA), Cysteine Lactose Electrolyte Deficient agar (Difco Laboratories, Detroit, Mich), Mannitol salt agar ((Difco Laboratories, Detroit, Mich), and Sabourad Dextrose Agar (Difco Laboratories, Detroit, Mich). A culture of each sample was done in triplicates. All plates were incubated at 37ºC for 24 hours in an incubator. Sabourad Dextrose Agar (SDA) plates used for the isolation of fungi were kept for 1week at room temperature. The colonies were sub-cultured to obtain pure colonies. Pure isolates of bacterial colonies were Gram differentiated and biochemically characterized and identified using the standard taxonomic schemes [19, 20]. The isolates were maintained in Nutrient agar slants in McCartney bottles and preserved in a refrigerator at 4^oC and for further analysis.

Antibiotic and antifungal susceptibility tests

The bacterial isolates and fungal isolates obtained in this research work were subjected to susceptibility testing using appropriate drugs. The susceptibility testing was performed by means of Kirby-Bauer disc diffusion method using the guidelines provided by[21]. The routine antibiotics used were; Ciprofloxacin (5µl), Ceftazidim, (30µl) and Streptomycin (10 µl), Gentamycin (10 µl), (Hardy diagnostic, USA). The antifungal isolates drugs used were Nystatin (25mcg), Fluconazole (25mcg), and Voriconazole (1mcg) (Hardy diagnostic, USA). The bacterial isolates were sub-cultured from agar slant on to a freshly prepared Nutrient agar (Oxoid, USA) medium for 18-24 hours at 37ºC to obtain a young culture. Each test organism was picked using a sterile wire loop and inoculated in 1ml of peptone water (Sigma chemical Co.Ltd, England.) and the organism was later plated on Mueller Hilton agar (Difco Laboratories, Detroit, Mich) plates. Cultures were done in triplicate. The plates were incubated at 37^0C for 24 hours. The results of susceptibility testing were read by measuring in milliliter (mm) the resultant zones of inhibition with a transparent metre rule. The Zone of inhibition (Z.I) values < 14mm is Resistant, Zone = 14mm is Intermediate, Zone ≥ 15mm is Sensitive. The values recorded were the means of three measurements of zones of inhibitions on the triplicate cultures their standard deviation of the mean was also calculated.

Statistical analysis:

The values of zones of inhibitions (Z.I) recorded were calculated from the means of three measurements of zones of inhibitions on the triplicate cultures and their standard deviation of the mean was also calculated (The (Mean± SD)

III. Results

Morphological and biochemical characteristics of bacterial isolates from roasted pork

The bacterial isolates obtained from the roasted pork samples were identified based on morphological and biochemical characteristics. They were; *Escherichia coli, S aureus, Vibrio* spp, *Salmonella* spp, and *Enterobacter* spp (Table 1).

TABLE 1. Morphological and biochemical characteristics of isolates from roasted pork

Gram Staining	Catalase test	Citrate test	Oxidase test	Coagulase test	Indole test	Motility test	Endospore	Urease test	Mannitol test	Glucose test	Lactose test	Sucrose test	Media	morphology	Bacteria isolates
- Rod	+	-	-	-	+	+	-	-	+	+	+	+	EMB	Green Metallic sheen	***E coli***
- Rod	+	+	-	-	-	+	-	-	+	+	+	+	EMB	Blackish brown	***Enterobacter* sp**
- Rod	+	+	-	-	-	+	-	-	+	+	-	-	MAC	Colourless	***Salmonella* sp**
- Rod	+	+	+	-	+	+	-	-	+	+	+	+	TCBS	Yellow	***Vibrio* sp**
+ Cocci	+	+	-	+	-	-	-	+	+	+	+	+	MAC	Pink	***S aureus***

Keys: + = Positive , - = Negative, EMB =Eosin Methelyene Blue Agar
MAC = MacConkey Agar, TCBS = Thiosulphate citrate bile salt agar.

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Morphology and microscopic characteristics of fungal isolates obtained in the study

The fungal isolates were identified in term of morphology and microscopic characteristics of fungal isolates obtained in this study. The two fungal genera identified were *Penicillium* spp, and *Aspergillus* spp respectively (Table 2)

TABLE 2. Morphology and microscopic characteristics of fungal isolates obtained in the study

Media	Morphology	Microscopic Characteristics	Fungal isolates
SDA	White filamentous colony developing dirty snuff brown spores with pale yellow to intense yellow felt beneath, forming yellow fringes around the spored colony. Bright yellow to dirty yellowish brown on reverse.	Conidial heads radiate then split into loose columns with age. Vesicle globosephalide borne on metulae. Spores globose with spines. Coffee-brown to black colour. Conidiophore stripes were smooth walled.	*Aspergillus spp*
SDA	Small white felt-like colonies becoming bluish-green has spores appear from the center outwards.	Brush-like conidial heads. One-step branching. Conidia in long chains, round conidiophores. Metulae in whorls of 3 – 5. Conidia resist disruption and are in long chains. Metulae longer than phalides.	*Penicillium spp*

Key: Sabourad Dextrose Agar (SDA)

Percentages of occurrence (%) of each isolates from roasted pork studied

A total of 31isolates comprising 26 bacterial and 5 fungal isolates were obtained in the study. The bacterial isolates were *E coli* (9), Salmonella (5), *S. aureus* (6), *Vibrio* spp (2) *Enterobacter* spp (4), and the fungal isolates were *Penicillium* spp, (3), and *Aspergillus* spp (2) respectively. The percentages of occurrences showed that the frequently isolated microorganisms in this study was *E coli* with the highest percentage of frequency of occurrence.29.1%, followed by *S. aureus* with 19.4%, while *Vibrio* spp and *Aspergillus* spp were observed with the same percentage of occurrence of 6.5% respectively (Table 3).

TABLE 3. Percentages of occurrence (%) of each isolates from roasted pork studied

Isolates	Frequency of Occurrences and Percentages (%)
Bacteria isolates	
E coli	9 (29.0)
Salmomella spp	5 (16.1)
S aureus	6 (19.4)
Vibrio spp**,**	2 (6.5)
Enterobacter spp	4 (12.9)
Fungal isolates	
Penicillium spp	3 (9,7)
Aspergillus spp	2 (6.5)
Total	**31 (100)**

Result of antibiotic susceptibility testing on bacterial isolates

Many bacterial isolates obtained in the study expressed multi-drug resistance pattern. It was noted that *E* coli-EA1 expressed resistance to Streptomycin and Ceftazidime with lower ZI of 9 ± 0.9mm and no zone of inhibition (NZ) respectively but being highly sensitive to Ciprofloxacin with wider zone of inhibition (ZI) of 37 ± 0.8mm. *Staphylococcus aureus*-EI1 was resistance to Ceftazidime and Streptomycin with ZI of 11±1.0mm and 10±1.8mm respectively. No zone of inhibition (NZ) was recorded for *E coli*-EA4 and *Vibrio spp*-EP2 against Streptomycin and Ceftazidime. Significantly, the results of antibiotic susceptibility of bacterial isolates from the pork showed Ciprofloxacin and Gentamycin antibiotics were highly potent against majority of these bacterial isolates from pork. The ZI ranged from 24±0.5mm to 37± 0.8mm for Ciprofloxacin and 17± 4.9mm to 35 ± 2.3mm for Gentamycin indicated high sensitivity of the isolates to these antibiotics (Table 4).

TABLE 4. Result of antibiotic susceptibility testing on bacterial isolates

Isolates numbers and codes	Ciprofloxacin ZI in mm	Gentamycin ZI in mm	Ceptazidime ZI in mm	Streptomycin ZI in mm
***E coli*-EA1**	37 ± 0.8	23.8 ± 1.7	NZ	9 ± 0.9
***E coli*- EA2**	28 ± 1.3	25.8 ± 3.3	12 ± 1.3	15 ± 1.2
***E coli*- EA3**	27 ± 1.2	32 ± 1.0	16 ± 1.0	8 ± 0.5
***S. aureus*-EI1**	27 ± 4.0	22 ± 0.3	11 ± 1.0	10 ± 1.8
***Enterobacter sp*-PA1**	26 ± 0.9	22 ± 4.3	NZ	18 ± 3.9
***Salmonella sp*-PI1**	29 ± 2.6	35 ± 2.3	NZ	10 ± 2.1
***E coli*-EA4**	33 ± 2.18	23 ± 2.50	NZ	NZ
***Salmonella sp*-PI2**	30 ± 2.5	30 ± 1.9	NZ	20 ± 0.5
***Enterobacter sp*-PA2**	28 ± 6.9	23 ± 3.3	11 ± 3.0	18 ± 1.8
***Enterobacter sp*-PA3**	24 ± 0.5	17 ± 4.9	9 ± 1.2	15 ± 3.5
***Salmonella sp*-PI3**	27 ± 2.3	21 ± 1.4	8 ± 0.5	13 ± 2.8
***S. aureus*-EI2**	26 ± 2.0	24 ± 3.8	NZ	18 ± 1.9
***S. aureus*-EI3**	26 ± 1.8	33 ± 2.3	9 ± 1.6	11 ± 2.1
***Vibrio spp*-EP1**	24 ± 2.2	21 ± 1.0	12 ± 1.0	NZ
***Vibrio spp*-EP2**	24 ± 1.3	20 ± 0.5	NZ	NZ
***Salmonella sp*-PI4**	24 ± 1.8	33 ± 1.4	NZ	16 ± 0.5
***S. aureus*-EI4**	27 ± 0.7	24 ± 2.8	15 ± 0.9	18 ± 0.5
***E coli*-EA5**	25 ± 1.4	21 ± 3.1	13 ± 1.0	13 ± 1.2
***Enterobacter sp*-PA4**	25 ± 1.3	20 ± 1.8	9 ± 1.3	12 ± 1.3
***Salmonella sp*-PI5**	32 ± 2.1	21 ± 0.6	8 ± 1.3	9 ± 0.9
***E coli*-EA6**	29 ± 0.8	32 ± 2.2	13 ± 1.2	19 ± 3.0
***E coli*- EA7**	30 ± 1.4	19 ± 2.5	13 ± 1.0	18 ± 2.5
***E coli*-EA8**	27 ± 0.5	19 ± 1.5	9 ± 0.5	19 ± 3.2
***S. aureus*-EI5**	27 ± 1.3	31 ± 0.9	17 ± 1.8	16 ± 0.2
***S. aureus*-EI6**	28 ± 1.6	23 ± 3.5	11 ± 2.1	21 ± 1.4
***E coli*-EA9**	35 ± 0.5	21 ± 1.8	12 ± 0.5	13 ± 1.6

Note: The zone of inhibitions (ZI) recorded above are expressed as the mean values of the zone of inhibitions of the triplicate cultures ± standard deviation.
Keys: ZI = zone of inhibitions, NZ= No zone of inhibitions

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Results on antifungal susceptibility testing

The results of the antifungal sensitivity test showed *Penicillium* spp-IA1 and *Penicillium* spp-IA2 were sensitive to Nystatin with Z.I of 29 ± 0.6mm and 25±1.1mm respectively. *Aspergillus ssp-BD1* was observed with a wider Z.I of 31±1.3mm to Nystatin and 32 ± 0.8mm to Voriconazole respectively. *Penicillium* spp-IA3 had ZI o**f** 10±2.3mm with Fluconazole (Table 5).

TABLE 5. Results on antifungal susceptibility testing

Number of isolates	Nystatin ZI in mm	Fluconazole ZI in mm	Voriconazole ZI in mm
***Penicillium* spp-IA1**	29±0.6	13±1.7	23±1.3
***Penicillium* spp-IA2**	25±1.1	19±1.2	23±0.9
***Aspegillus* spp-BD1**	31±1.3	25±0.9	32±0.8
***Penicillium* spp-IA3**	25±2.2	10±2.3	18±1.5
***Aspegillus*spp-BD2**	19±1.1	17±1.1	18±0.6

Note: The zone of inhibitions (ZI) recorded above are expressed as the mean values of the zone of inhibitions of the triplicate cultures and standard deviation.
Keys: ZI = zone of inhibitions,

IV. DISCUSSION

The recovery of microorganisms from pork samples screened showed degree of microbial contaminants. The predominant organism of which was E *coli* and closely followed by *S. aureus* many researchers reported difference microbial contaminants from pork as well as different organisms with the highest percentage of frequency of occurrence. For instance, a certain work reported the presence of bacterial pathogens in pork with *Staphylococcus aureus* as the predominant organisms found with the highest percentage of frequency of occurrence[22]. Other work reported the presence of *Salmonella* in the pork screened in their work with the highest frequency of occurrence[23]. *Staphylococcus aureus* is a member of the normal flora of the respiratory tract, gastrointestinal tracts and human skin. However, the public health implication is that *Staphylococcus aureus* enterotoxin producing strains of *S. aureus* is a leading cause of food intoxication as it can produce extremely potent gastrointestinal toxin [24,25]. *Escherichia coli* is a member of Enterobactericeae found in gastrointestinal tract of humans and animals[26]. Their presence in the ready- to eat meat is an indication of fecal contamination either direct or indirect means. Their presence of these bacteria and fungi in the ready- to eat pork samples may be due to the unhygienic status of the slaughter houses, which portrays that the pork was poorly prepared and even the prolonged exposure to the surroundings can bring possible contaminants to the meat products. The health status of animals prior to slaughtering, and prevailing circumstances in the slaughter contributes to the quality of meat from such animals[27]. Other pre-disposing factors of contamination of the m eat that could warrant the presence of these organisms could also be processing points, handling and selling [22]. Some researchers in their work asserted the fact that .the wide spread distribution of the meat product makes the consequence of contamination with food poisoning microorganisms more serious[28].

In this study, it was observed that bacterial isolates exhibited varied ZI expressing either sensitivity or resistance. Some isolates expressed resistance to multidrug. Some *Salmonella s*trains formed resistance against Ciprofloxacin, Ceftazidime, Gentamicin and the Streptomycin. Similar reports were documented [29,30]. A study reported many strains of *Salmonella* that were resistant to Ceftazidime, Gentamicin, Chloramphenicol and Ciprofloxacin[31]. Another study reported multi-antibiotics resistance and extended spectrum beta-lactamases (ESBLs) production by *Salmonella* species [32]. The multidrug resistance patterns observed in the study especially from *S. aureus isolates* is therefore of serious concern since it could be a source of food borne disease outbreak in a community at large [33,34]. *Staphylococcus aureus* has been recognized as a major human pathogen. *Staphylococcus aureus* has been found to be the most clinically important species, with broad presence in nature. It been recognized as one of the most common cause of human infections, which include bacteremia, skin infections, wound infections and food borne infections. Many researchers have discovered that *Staphylococcus aureus* have rapidly developed resistance mechanisms against many antimicrobial agents[33-35]. Some strains of *E. coli* in this study expressed resistance to two or more antibiotics tested. Similar report also was made by researchers who in their work observed *E. coli* isolates expressed resistance to the largest number of antimicrobial [35].However, quite different what was observed among bacterial isolates, none of the fungal isolates obtained in this study was resistant to the chosen antifungal agents except *Penicillium* spp-IA3 which had smaller ZI with Fluconazole. High sensitivity of fungal isolates to the antifungal drugs was reported by other researchers[36]. The prevalence of antimicrobial resistance is very rampant among bacterial isolates. Indiscriminate use of antibiotics in both medical and veterinary medicine has resulted in wide spread antibiotic resistance and development of antibiotic resistance genes [37]. However, Ciprofloxacin and Gentamycin antibiotics were highly potent drugs against majority of these bacterial isolates from pork. Therefore, Ciprofloxacin and Gentamycin antibiotics can be drugs of choice while treating infections caused by these isolates

V. CONCLUSION

It was observed in the study that ready – to- eat pork samples screened harbored pathogens of which some of them expressed resistance to common routine antibiotics. The presence of these organisms could possibly linked with the low level of sanitary practices from the processors and producers as well as handlers of this meat product. Consequent upon this, emphasis is laid on good sanitary and hygienic practices on the parts of pork producers, the consumers should avoid indiscriminate eating of meat sold in the open that is not properly reheated. Awareness and enlightenment campaign is vital to discourage self – medication, as well as enforcement and caution of laboratory

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examination by the clinicians in the study area before antibiotics prescription and administration.

Significance Statement

This study discovered that pork samples screened had some pathogenic isolates as contaminants and the isolates exhibited varied degree of resistance to routine antibiotics. Some strains of bacteria expressed multi- drug resistant (MDR) pattern with h*igh* resistance to Ceftazidime and Streptomycin antibiotics. This is a potential threat to human's health through food borne transmission. The result of this study has given data-based information, which will therefore be useful and beneficial in formulating antibiotic policy which will serve as an important tool in the management of infections caused by these isolates. Moreover, since microorganisms developed different strategies to counter the effects of antimicrobial drugs; the identification of the resistance mechanisms is vital. This calls for further research in finding out whether this resistance phenomenon is mediated by genetic mutation or plasmid transfer among antibiotic resistant microbial strains. Thus, the discovery and design of new antimicrobial agents will be arrived at.

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Conflicts of interest: We state that this research study has no potential conflicts of interest.

Acknowledgement: We wish to thank staff of Microbiology laboratory, University of Uyo, Akwa Ibom State, Nigeria for their assistance and cooperation.

Contributions of Authors

Dora Imefon Udoh designed the work and wrote the manuscript, Uduak Richard Obot did field work, processing of the samples, laboratory analyses were carried out by all authors while analysis of data was done by Bassey Bassey Etang.